AGE AND HISTAMINE METABOLISM

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C O N T E N T S

Prefactory Note

The work described in Section 4 was done in collaboration with Dr. E. Geiringer who was responsible for the vivisections.

1. INTRODUCTION

General Introduction

There are two approaches to the problems presented by the aging of a living organism. The first, the geriatric approach, is concerned with measures to alleviate the more unpleasant consequences of the changed metabolism of old people. Such an approach is necessitated by the human problems confronting clinicians. It might be thought that the results of such work would be useful in showing why the metabolism of the old differs from that of the healthy young adult.

Such changes as are observed are, however, generally caused by two factors, namely the aging of the organism as such and secondary pathological effects. Death due to the first factor alone may occur in a few patients according to Moor (116) who described it as "metabolic failure" or "death due to pure old age". Clinically such cases are difficult; Moor says, in effect, "whatever you do, the end is inevitable". As Korenchevsky (86) points out, the commoner secondary effects are more amenable to treatment. Humanitarian feeling and relative ease of approach have therefore combined to recommend methods of geriatrics to many workers.

The more fundamental approach is that of gerontology which seeks not to alter a pathological condition but to find out why the organism becomes less efficient with the passage of time. In the

last analysis this decrease in efficiency must be caused by an alteration of the chemical or physio-chemical structure of the organism or the nutrients available to it. Lowry and Hastings (101, p. 107) remark, "Attempts to delay or circumvent the changes which decrease the efficiency of the organism await the chemical and physiological description of the aging process". The changes ultimately looked for will be slight for, as Lansing states (90), "Aging involves changes in cells of a subtle nature, changes which cannot be detected by direct microscopic observation. Progress in an understanding of the physio-chemical changes in senescent cells will depend upon acquisition of reliable data on specific changes in cells which perhaps ultimately will be blended into a coherent system".

Many theories and definitions of aging have been advanced (see for example the discussion on "What is Aging", 176), and as a result the field is somewhat confused with no clear indication as to the best directions research could take. It would seem necessary therefore to review some of the ideas held and to state the author's approach to the theoretical problem.

Theories of Aging

The idea, based on superficial observations, that aging is due to the dehydration of the tissues has been held for centuries. It is not altogether

supported by recent evidence (20, 157) but probably engendered the concept described by Dhar (38), Kopaczewski (84) and others that the colloidal constituents of the body decrease in reactivity and solubility with age. The analogy with inanimate colloids is invoked to support such a thesis but is seen to break down when it is remembered that the body colloids are being continually decomposed and resynthesised. This state of flux may be necessary to maintain enzyme proteins in an active state (72).

Lansing (89), in an extension of this concept, suggests that the accumulation of calcium at the cell boundary interferes with the transmission of food and waste. No one has yet demonstrated such interference, and Reiner (127) concludes that, even if they occur, alterations in cell permeability are not fundamental to aging.

Moulton (118) measured water, organic matter and fat throughout the life-span of cattle, swine, guinea-pig, cat, dog, rabbit, mouse and rat. He found values became approximately constant at periods after conception, representing about 4.3 to 4.6 per cent of the total life-span in all species but the cat where the figure was 3.9 per cent. On this basis he suggested the concept of "chemical maturity" of tissues.

Asdell (7), whilst agreeing that "chemical maturity" has physiological significance, pointed out that the age of death has to be selected some-

what arbitrarily to calculate the percentage of life-span after which chemical maturity occurs. Horvath (74) showed that the age of chemical maturity is dependent on the constituent whose concentration is used for the calculations. Nevertheless, the concept is important as it throws into relief the necessity of considering cells and tissues as labile chemical systems and the consequent need to investigate tissue constituents throughout life. As will be shown below, the fact that some bodily constituents do reach and maintain a constant level through a large proportion of their life-span is extremely interesting from a theoretical view-point.

Various authors have made mathematical approaches. Simms (150) studied mortality and debility rates and concluded, "The theory that senescence results from a random accumulation of degenerative changes is not supported by these findings" as this would give a linear, not the observed exponential variation of mortality with age. Such a relationship is consistent with "an accumulation of degenerative changes such that the rate of change at any age depends upon the amount of accumulated change" (that is, an auto-calalytic process).

A most significant study of the steady state was made by Burton (21). The steady state is distinguished from equilibrium in that initial reactants are fed into the system and end products are removed in such a way that intermediates are

maintained at a constant level. Unlike the equilibrium state, this requires an alteration in energy content. Such a steady state is found (at all events to a first approximation) in the living organism.

Burton showed that if the activity of an intermediate is changed the system (left subsequently to itself) will revert to the initial condition. If, however, the activity of a reaction catalyst is altered the system may take up a new steady state. Now in the living organism, some of the steady state reaction chains are involved in synthesising some of the catalysts, that is enzymes, so that in some cases alteration of intermediates will indirectly alter the activity of enzymes, if their synthesis is materially impaired before the return to normal. It should be remembered that, owing to their complexity, proteins may be altered very slowly by slight successive changes in structure.

Some evidence for such change is provided by the work of Noyes and Faulk (122) who indicated that enzymes became more specialised (and possibly therefore more efficient) during growth and then declined towards their initial condition with age. (In this case functional identity does not necessarily imply chemical identity.) The work of McCay et al. (104) on delayed maturation bears a similar interpretation. During the period of stasis the immature enzyme systems degenerate to a certain extent (dependent on

the length of growth arrest); when allowed to mature the system as a whole is not so efficient and breakdown should occur much sooner. Such a result was actually observed as rats retarded for 300 days lived 535 days after maturity, whereas those retarded for 1,000 days lived only 138 days after maturity. The retarded animals seemed much more susceptible to stress than normal adults. Reiner, too, (127) concludes from work on tissue respiration that protein was altered in amount, state or both.

It is therefore possible that by a very gradual process the functioning of the organism's enzyme systems may be impaired due to variation in the concentrations of initial or intermediate reactants. Burton states that "The stability of biological systems is, in general, of a limited kind. A fluctuation from the steady state is compensated by restoring forces if it be small, but fluctuations greater than a certain amount are followed by disorganisation of the system which may be temporary or permanent".

Of course, such a model system is only a crude approximation to an organic system, and the model would appear more sensitive than the reality. Clearly the method could be extended and the present author has tentatively examined the cyclical system.

$$x + e_1 \longrightarrow y + e_1 + e_1$$

$$y + e_2 \longrightarrow x + e_2 + e_2$$

where x, y are substrates and e_1, e_2 enzymes.

That is, an enzyme undergoes auto-synthesis to increase its own substance and also forms a diffusible substrate for another enzyme, undergoing auto-synthesis whose diffusible product acts as substrate for the first enzyme (following Hinshelwood, 72).

Although unlikely in this simple form, it is mathematically equivalent to a much larger cycle of processes. By consideration of the rate differentials it can be shown that when the concentration of x or y is displaced the system may oscillate about the original values and gradually return to them or it may gradually move away from the initial state, depending on the values for the reaction constants. In combination with Burton's system this would allow for a gradual change through adult life, terminating in a relatively sudden breakdown as reaction-constants attained critical levels.

Reiner and Spiegelman (128) have extended the study of the steady state to the thermodynamics of the system. It is to be hoped that the promised investigation on "disturbed steady states" will appear, though, as they point out, "the solution of the non-stationary forms presents a mathematical problem of great complexity". A. R. Ubbelohde (164), in a popular article, has emphasised the philosophical importance of thermodynamic studies in relation to aging: unfortunately he does not appear to distinguish clearly between equilibria and steady states.

The concept that aging is due to the disturbance of homeostasis is not new. At a cellular level Vogt and Vogt (116) suggest that the gradual diminution in the number of brain cells would lead to the death of the organisms when the safety margin has been reduced to zero, and the concept is expressed generally by Cowdry (27, p. 84). Lansing (90) states that "aging is a problem of cellular change - of unfavourable change in the ability of protoplasm to maintain itself by synthesis", and a recent editorial in the "Lancet" (175) says that "it seems certain that old age is a general failure of homeostasis". Euler, like Lansing, emphasises the importance of proteins in the statement (42), "Many of the low-molecular ergones influence growth but as their formation and functions depend on governing regulators, they cannot be directly connected with the primary causes of aging. However, the governing hormones are high-molecular proteins: their chemical changes seem not to be reversible and of their regeneration we do not know more than we know in the case of the regeneration of brain or nerve cells". Shock (149, p. 473), reviewing the subject, concludes that aging is due to the failure of homeostatic mechanisms.

It is not doubted that in the body given systems are homeostatically controlled until late in life, if not until death: it is obviously essential that vital metabolites should be held at

suitable levels. What is suggested is that the buffering system which maintains such constant levels is gradually depleted. The depletion of reserves causes the organism to become less and less able to withstand stress (in its widest connotation) in later life (see, for example, Bourliere,(16) and Smith and Shock, 154). Thus the aged organism is more likely to succumb to pathological conditions whether infectious or metabolic, and most old animals will die from such causes. In this connection may be mentioned Benedict and MacLeod's (11) observation, in the case of three rats, that basal metabolism rose throughout life, then fell sharply within a day or two of death. This may have been due to a failure of homeostasis subsequent to starvation.

The possibility remains that some organisms will escape a testing of their depleted reserves until these are completely exhausted. Mora and Greene (117), on the basis of human goitre studies, suggest that the aged subject avoids unnecessary systemic strain and thus the danger of succumbing to a critical stress is lessened and "metabolic failure" is more probable. The organism at this stage may be said to be living on a metabolic knife-edge. It is in the light of these considerations that Moor's (116) concept of metabolic failure (if such it is) is to be viewed. Statistical information of the number of such cases would be of interest: Moor states he meets about one a year but does not

indicate the size of his practice.

The view of aging here put forward is well epitomised by Albertini (4) in the definition "aging is a decrease of adaption as a consequence of a loss of tissue and functional reserves". How does this loss of reserves come about? There are three possibilities. The first is that under usual conditions the tissues are not supplied with nutrients at the necessary rates to maintain their original (or unattained ideal) state; this implies that there exists the possibility of so adjusting the diet both as to kinds of food and as to rate of intake as to supply the tissues optimally. However, the needs of the individual tissues and the ability of nutrients to penetrate to the required points may so vary that to satisfy the entire body would be impossible.

Secondly, the variation from the original (or ideal) state may be due to the impact of stresses. Dempsey (36, p. 406) in effect suggests this possibility on the physiological plane. We have not sufficient information to agree with Reiner (127) that "all those environmental fluctuations which are observed are too small to tax the homeostatic mechanisms of the organism and its component cells". We do not know what will affect adversely the future functioning of an organism.

It is of interest in connection with the role of stress to note that Simms and Stolman (151) observed "senile" changes in young persons that had

been subject to severe disease. The greater incidence of thyroid disease in regions of greater temperature fluctuation (Dempsey, 36, p. 408) may also be relevant. Hines and Knowleton (71) reported that changes occurring in rat gastrocnemius muscle after inanition resembled those occurring in old age. Lewis (93) points out that those venules subject to the greatest environmental changes are most likely to lose tone and ability to react to histamine and adrenaline.

The third possibility is that under the most favourable conditions practicable the proteins are incapable of full and perfect resynthesis, a possibility which at the present state of knowledge cannot be tested against any facts. Reiner (127) states that "failure of crucial enzymes in a cell to be reproduced or to reproduce themselves would account for the known facts". More fundamental information concerning the physics and chemistry of biological macro-molecules is the greatest need in gerontology as in many other fields.

These three possibilities are well-paralleled by Roger Bacon (McCay, 102, p. 146) as available food stuffs, general environment and a limit set by God respectively. It is obvious that they are closely interrelated.

Thus to understand the aging process fully necessitates information on the changes of metabolite concentrations throughout the life-span and on the

reasons for any change; for "states favourable to virus growth, to a lack of tissue restraint or to tissue stimulation cannot be ascertained until we possess bio-chemical and bio-physical information of cell life at various periods in the life of the organism as a whole in order to contrast chemically, through these data, periods in the life-span of tissues characterised by relative resistance or susceptibility to change of a malignant order". (MacNider, 106). This author goes on to state that "basic research of a chemical order ..... has certainly lagged in its applied interest," and as recently as 1951 the "Lancet" (175) pointed out that "this is a field where even the ground work has yet to be laid".

It was against such a theoretical background and as a preliminary to a programme of studying the bio-chemistry and physiology of the skin and their changes with age that the present investigation into the histamine content of skin in the albino rat was undertaken.

Histamine and Skin

The first evidence that skin contained histamine was obtained by Harris (67) in man and the cat, following the work of Lewis. Since then work has shown that histamine is present in mammalian skin in considerable quantity (Table 1). Alexander (5) found that about 70 per cent of the histamine in mice was in the skin. Work on the frog (60) and

giant lizard and turtle (14) skin indicates that histamine is not present or active in cold-blooded animals, at all events to such an extent.

Harris showed that in human skin the epidermis contained most of the histamine, 24 microg./g. compared with 4 microg./g. in the dermis. This work unfortunately appears neither to have been confirmed nor extended. Lewis (93) suggests that living epidermal cells are the important tissue for pain and the triple response. Histamine may be associated with the smaller arteries, according to Miles and Miles (113), and West and Riley (169) indicates that such histamine may be contained in the mast cells.

The site of the skin sample appears to influence the histamine content (Table I). Harris found that skin from the human thigh had a higher content than that from the breast (though it should perhaps be noted that the thigh samples were male, the breast samples female). In the case of the rat values for the abdomen appear higher than the back; the figures in the present paper are all obtained on samples from the flank and agree best with those from the back. Again, the values for cat's hind legs and abdomen appear higher than for the flank (though this value was obtained one hour after burning the opposite flank). Feldberg and Miles (46) studied the distribution of intravenously injected dye after treating guinea pigs, with 48/80;

a larger accumulation is associated with a higher histamine content except in the case of the paws: detailed figures have not yet been published. Ears, snout and eyelids show the biggest effect, the fore part, external genitals and nipple area also showing a moderately high accumulation.

Lewis (93) remarks on the localisation of "blushing" phenomena to areas normally exposed to the light. Variation in skin histamine may explain this. The reason for such variation may well be, as has been suggested, that these areas are subject to more frequent stimulation. The fact that the rat's abdomen has a higher content than back or flank is suggestive. Andrews (6) finds that the abdominal epidermis in the rat is thicker (22 microns) than dorsal tissue (13 microns). Assuming that the dermis does not change in a parallel manner (and this is by no means certain), the differences in content may be related to the larger proportion of histamine-rich epidermis in the abdominal skin.

Pellerat and Murat (124) noted that the local variation (samples about 5 cm. apart) was negligible in human beings. Care has been exercised throughout this work to obtain samples half-way between the dorsal and ventral mid-lines, between shoulder and hip.

Variation of histamine content also occurs with age. Harris found that infants 6 - 10 weeks premature had a high (18 microg./g.) content of skin

from the breast and back. Young infants also had a high content in the fore-skin, but Harris suggests this may be due to the low fat content. On the other hand Feldberg and Schilf (49) note that new-born ape skin contained only 2 microg./g. The present work shows that foetal rat skin is probably low until within a few days of term, when it rises sharply to high values at birth.

Pellerat and Murat (124) noted that old individuals (over seventy years) had lower (13.4, 11.4,2.0 microg./g.) values than normal (17.3 - 23.7). This may be associated with the lower reactivity of old skin: for example, Jankowski (77) irritated skin mechanically and noted that an old subject showed much less reaction than younger human beings: Shock (149, p. 423) reports that aged human skin vessels have a decreased ability to dilate. The figures given by Rocha e Silva (132) for rabbits indicate that histamine content may be low in the heaviest (? oldest) animals. His results show no clear relationship between histamine content and thickness of whole skin.

Rocha e Silva's results also show that young (50 - 150 g.) rabbits have a high content. This is analogous to the finding of Marshall (107) that young rats have a high skin histamine content (Table II). The present work confirms this observation.

Short term fluctuation of the skin histamine

has been shown to be small. Nilzen (121) took samples from the forearm of men at intervals of 3 to 16 days (in one case, four samples over the course of a month) and showed a maximum variation of 15 per cent. Emmelin (40) found a slightly greater fluctuation in rats and guinea pigs. Plasma from these animals exhibited a similar constancy.

The effects of histamine on the skin were well described by Lewis and Grant (94). Three phases can be distinguished, referred to collectively as the triple response. First a reddish-purple spot at the site of injection, then swelling and a more suffused reddening. These are shown to be due to immediate dilation of the capillaries, oedema, and dilation of nearby arterioles respectively. Such effects are elicited by pricking the skin through a drop of histamine solution containing 330 microg./ml. Lower concentrations can be infused (167) or injected (78) to give similar effects.

Of these effects the dilation of the blood vessels is well established. Some workers have considered that the increased permeability of the capillaries is not necessarily mediated by histamine under physiological conditions. Cullumbine (30) stated that neoantergan did not prevent the oedema produced by burning rabbit's abdominal skin, although it was effective against an increased spread of Trypan Blue after histamine

injection. The latter observation confirms that of Rocha e Silva and Dragstedt (135) who suggested the Trypan Blue technique as a test for histamine liberators.

Dekanski (35) found that leukotaxine (believed by Menkin (110) to be responsible for increased capillary permeability) increased skin histamine. He considered that effects due to leukotaxine were mediated by histamine, but points out that oedema is due in all probability to a failure of lymph to increase its flow commeasurately with that of blood: increased permeability is therefore an unnecessary hypothesis. Recent work by Matoltsy and Matoltsy (109) indicates that histamine induces phagocytic action in the capillary endothelium.

Histamine is said to cause pain. Lewis (97) considers that the pain caused is slight (described as itching), and Emmelin and Feldberg (41) state that a mixture of histamine and acetylcholine in high concentration gives more pain than either drug separately. On the other hand, Rosenthal and Minard (141) and Lambert and Rosethal (88) and more recently Euler and Astrom (43) and Rosenthal (140) have presented evidence indicating that histamine is released by electrical stimulation of nerves. The cumulative evidence that histamine is involved in some nervous reactions is strong; the secondary arterial dilatation referred to above is a case in point. We have no conclusive evidence, however,

that histamine is concerned with the sensation of pain. The relatively high concentration to be applied to cause pain may be due to poor penetration of the drug to the site of action. The fact that anti-histamines are to a certain extent anti-acetyl-cholines complicates the problem (129).

Lewis and his associates (92 to 95) compared the action of histamine in the skin with the result of mild irritation (such as drawing a blunt instrument firmly over the skin) particularly in cases of urticaria-factitia. The reactions were found to be identical in appearance and time relationships, and this identity led Lewis to suggest that histamine or a similar substance is primarily responsible. Their results, together with those of earlier workers indicate that, while the initial capillary dilatation was a local effect, the arterial response was mediated by a nervous reflex.

Analogy led many workers to attempt to show histamine release in a number of allergic and traumatic conditions. Either blood histamine has been shown to rise or skin histamine content shown to change. The first method involves considerations outside the scope of this review; a good summary of previous work is given in Nilzen's (121) monograph.

The direction of change in the skin content will depend on the sum of a number of factors. The extent to which stasis occurs will determine

not only how quickly histamine will be brought in or removed, but also the quantity of precursors, products and others associated metabolites transported (Lewis (93) suggests that local metabolic rate is a factor in the dilatation of blood vessels). The integrity of enzyme systems which produce, release, destroy or conjugate histamine and especially their relative amounts before and after stimulation or injury will influence histamine content. Thus changes in histamine content should be related to such factors, but the available information is too limited for this approach to be fully exploited. Histaminase was not detected in dog skin (13), but more recently Granroth and Nilzen (59) showed that it was present in guinea pig, rabbit and human skin, but absent or low in cat skin. Feldberg and Paton's (47) observations suggest that cat skin is low in histaminase. A few experiments by the author indicated that there was little or none in rat skin. No reliable data are available for the presence of histidine decarboxylase in skin. However, Lewis (93) implies a continuous production of histamine (H-substance), and Dekanski (33) showed that the total extractable histamine in mice increased after burning.

Tarras Wahlberg (159) related the change found after ultra-violet irradiation to blood values. The rabbit, with a high blood content, showed an increase, the cat, whose blood histamine is very

low, a decrease. Tarras Wahlberg's observations on the rabbit agree with those of Kawaguchi (80) on the guinea pig, though Ellinger (39) had previously found little qualitative evidence of a change with this species. Kawaguchi also noted that non-irradiated (ventral) skin showed an increase. However, Loos (98), who burned one ear of a rabbit, found an increase in the burnt but not in the unburnt ear.

Harris (67) showed that, in cats, histamine decreases after a moderately severe burn. Dekanski (34) found that the direction of the effect was temperature-dependant: at 60°C. the skin histamine rose, at 80°C. and 140°C. it fell. The same author (33) showed that the total histamine in mice immersed in water at 60°C. rose to about twice the value; the skin histamine rose more, proportionately. These findings suggest that the appearance of histamine is mediated by an enzyme system destroyed above 60°C.

Cold (ethyl chloride freezing) will also liberate histamine from the skin of human beings and guinea pigs (124). These authors also showed that in tubercular allergy the human skin values were below normal. Horton, Brown and Roth (73) found an increased gastric secretion in patients with cold allergy when exposed to this irritant. Other allergic and anaphylactic symptoms have been correlated with histamine release, following Lewis (93).

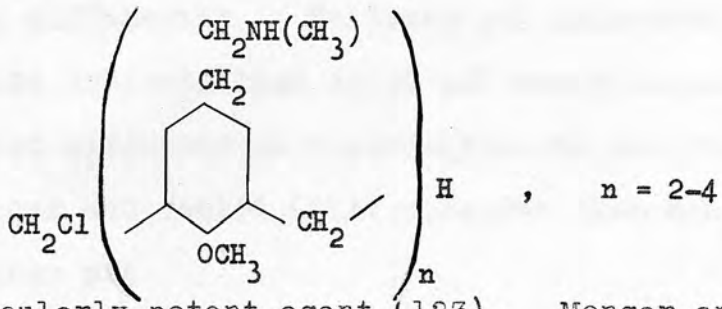
Riesser (131) showed a great increase in rabbit skin histamine after shocking with ox-serum. Schild (145), working with isolated guinea pig tissues, found that skin liberated 0.1 - 0.2 microg./g. More recently Mongar and Schild (114) reported 0.3 - 0.7 microg./g. using a larger sensitising dose of egg albumin. Nilzen (121), in an admirable monograph, described a decrease in human skin content after mechanical irritation and found that dermatographic patients showed a fall somewhat earlier than normal subjects. More recently, Feldberg and Schachter (48) and Schachter and Talesnik (144) have found that horse serum and eggwhite will release histamine in the isolated perfused skin of the cat without sensitisation, but dogs must be sensitised. Rats behave like cats to eggwhite.

Work has been done on the effect of chemical compounds on skin histamine. These are of two classes:- substances which irritate the skin, analogous to mechanical or anaphylactic disturbance (Lewis, 93) and substances which release histamine, presumably by some interaction with the site of adsorption. The connection between these is discussed by Mongar and Schild.

Loos (98) demonstrated an increase in histamine after treating the rabbit ear with cantharidine plaster. Similar observations have been made by Haas (64) who tested a range of substances on rat skin. He divides such substances into those which

injure cell nuclei and cause an increase in skin histamine, and cytoplasmic poisons which cause a slight decrease.

More recently, Macintosh and Paton (105) studied the effect of injecting a series of aliphatic diamines and other bases, and found that histamine was probably released from skin or muscle. Skin perfusion experiments by Feldberg and his associates have confirmed the release from skin by such histamine liberators (47)(communication to the Physiological Society Meeting, January 3rd 1953) and these workers have shown that 48/80



, n = 2-4

is a particularly potent agent (123). Mongar and Schild (114) showed that 48/80 releases about the same amount of histamine as anaphylactic shock in guinea pig tissues.

That histamine plays an important part in the reaction of injured skin can be scarcely doubted, though its exact position in a given chain of reactions is by no means certain. How it arises in the skin is still unknown. The increase in unaffected skin after injury (34, 80, 159) suggests that it is transported there, and Dekanski's (33) observations are not inconsistent with this. Gotzl and Dragstedt (57) feel that their results on

thyroidectomy and hyper-thyroidism indicate that histamine is produced locally in the skin. Feldberg and Talesnik (50) find that dog skin deprived of 85 per cent of its histamine by 48/80 recovers only slowly. On this basis they suggest that restoration only occurs by newly formed cells. One wonders what the effect of injected histamine would be on the histamine content of these tissues.

These workers go on to suggest that their results do not favour Lewis'(93) concept of histamine as a defensive agent. However, 48/80 and injury might work differently. Feldberg and Schachter's (48) results indicate that 48/80 and anaphylaxis are somewhat different in their action in the dog, though Mongar and Schild (114) consider them similar in the guinea pig.

Since histamine is present in such large quantities and yet exerts no effect and since it can be liberated by the action of other bases, it would appear to be bound, presumably to proteins. It is of interest to note that proteolysis is a common feature of many of the conditions in which histamine is believed to participate. The author has found that rat skin will take up histamine in vitro on being ground with sand.

Skin has been the subject of a number of gerontological investigations (2, 6, 20, 28, 81, 82, 100, 156, 171). This tissue has the disadvantage that it is polyfunctional, so that changes in one functional region may be masked by reverse changes elsewhere or simply lost in the larger bulk of the complex tissue. On the other hand it is readily examined and biopsy specimens may be obtained without too violent interference with the subject's general metabolism. The importance of this is clear from the theoretical considerations given above.

The following investigation falls into two main sections:- the determination of histamine values with age, and the changes of skin histamine after stress. These last results, together with some ancillary observations indicate that rats weaned at 21 days may undergo stress owing to immaturity.

2. MATERIAL

The Wistar WAG strain of rats was used for these experiments. The animals were all bred by sibling matings from one litter obtained from Glaxo Laboratories in May 1950. The importance of using pure strains for gerontological research in order to eliminate genetic variation was pointed out by Loeb (97). In a few cases litters showed ring constrictions of limbs and tail but such animals were discarded and the parents not used for further breeding. Apart from this all the animals appeared normal and healthy. Rat cake as supplied by the North-Eastern Agricultural Co-Operative Society (14 per cent rat cake) was freely available to the animals at all times. This cake has been shown by the Rowett Institute to be a sufficient diet for rats. Nevertheless, as a safeguard, this diet was augmented by wholemeal bread and milk three times a week and by raw cabbage or ABIDEC vitamin preparation. Water was always freely available. The cages were bedded with sterilised wood wool and so constructed that faeces and urine fell through the bottom. The animal house was fitted with ultra-violet light sterilisers, and the temperature was kept between 18 and 24°C.

Young animals were weaned at 21 days by removal of the mother. Sexes were separated at 42 days.

3. THE VARIATION OF SKIN HISTAMINE WITH AGE

Methods

(i) Skin Sampling

The animals were lightly anaesthetised with ether and, after removing the hair with scissors over an area of the flank, a piece of whole skin, weight 0.1 - 1.0 g., was removed and transferred to a dish containing 0.9 per cent sodium chloride solution for histamine assay. It was thought inadvisable to use chemical depilatories as they might cause local injury leading to a change in the histamine content of the skin. (Rocha e Silva (132) considered that other workers had found higher values than he in rabbit skin because they used chemical depilatories.) Care was taken as to the anatomical position for reasons given above (p. 13, para. 3). Skin samples were removed from the flank half way between the dorsal and ventral mid-lines.

In the cases where foetal values were obtained the pregnant females (judged near term) were opened by a ventral mid-line incision, and one uterine horn emptied of foetuses. Samples were taken from both flanks of each foetus. In one case (six foetuses about four days before birth) all the samples were pooled for estimation; in the other case (five foetuses about two days before birth) one piece from each animal was pooled for one assay, the other piece from each animal being pooled for a second assay. The age of the foetuses was estimated from the date of birth of the other half litter. The

problems attached to this are discussed below

(ii) Extraction and Assay of Histamine

The piece of skin was dried between filter paper and weighed within ten minutes of excision. This wet weight was used to calculate the histamine content (as base) per gramme. The sample was then minced with scissors into a mortar and ground with two or three times the weight of quartz sand for about one minute. The mixture was washed into a beaker with 25 ml. water and an equal volume of ten per cent trichloracetic acid added. (In some cases the mixture was washed over with saline and centrifuged. The supernatant liquid was precipitated with 25 ml. trichloracetic acid and the residue suspended in 5 ml. trichloracetic acid plus 5 ml. water. The resulting solutions were treated and estimated separately, and the sum of the contents taken as the histamine content. The residue contained 10 - 30 per cent of the histamine.) After standing for some hours (generally overnight) the solutions were filtered through Whatman No. 41 papers, and the residue washed three times with 5 per cent trichloracetic acid.

The clear filtrates were then boiled with the addition of 10 ml. concentrated hydrochloric acid for 1½ hours (allowing the volume finally to decrease to about 10 ml.). The solution was washed into a boiling tube with 10 ml. absolute alcohol and evaporated to dryness under reduced pressure.

The residue was twice dried off with 7 to 8 ml. of alcohol and then extracted three times with 2 ml. portions of physiological saline (or water if saline had previously been used). The extracts were filtered through a micro-Hirsch funnel and the filtrate neutralised with 0.2 N NaOH, using Thymol Blue as an external indicator (it was found convenient to use Bromcresol Green pH 3.6 - 5.2 initially). The neutral solutions were made up to 10 ml. though subsequent dilution to 20 - 100 ml. was usually necessary. This method follows Code's (25) modifications of Barsoum and Gaddum's (9) technique for preparing extracts for histamine estimation.

Assays were performed on a piece of guinea pig ileum suspended in a 2 ml. bath of atropinised (10^{-6}) Tyrode's solution under a load of about 1 g. (Pieces of gut were generally found to be satisfactory after up to one or two days' storage in glucose - Ringer's solution in the refrigerator at about 2°C.) The substance estimated resembled histamine in that contractions to both substances were sharp and relaxation occurred before or very shortly after washing out (40 seconds after introducing the dose). In some cases mepyramine maleate was shown to abolish responses to both substances at a concentration of 5×10^{-8}; subsequently the responses recovered in a parallel manner. As also the extraction method employed is to a certain extent selective for histamine (destroying substances

unstable in acid) it was considered that the substance was histamine.

Experiments to check the recovery of histamine from aqueous solutions gave 90, 75 and 98 per cent recovery of 6 microg. of histamine; recoveries of 80, 93, 92 and 85 per cent were obtained when 100 microg. were added to about 0.5 g. quantities of skin containing 13 - 20 microg. per gramme. All figures in this paper are given in terms of histamine base assuming that histamine acid phosphate contains one-third of its weight of histamine base.

Whole rats were used for histamine assay after being killed with ether. The results of Koshtojang, Rivkina and Mitropolitanskaya (quoted by Gaddum, 54) indicate that the histamine content obtained in this way may be high. The bodies were minced by means of an Atomix in a suitable volume of 5 per cent trichloracetic acid. After standing overnight the mixture was filtered at the pump on No. 42 Whatman papers. The residue was thoroughly washed with 5 per cent trichloracetic acid. The filtrate was diluted to 100 to 500 ml. with 5 per cent trichloracetic acid and aliquots taken for assay. The aliquots (1 - 10 ml.) were diluted to about 20 to 25 ml. with water, and 10 ml. concentrated hydrochloric acid added. The solutions were boiled and treated as above.

Digestion of the residue with approximately 3 N hydrochloric acid for 1½ hours and treatment of the

digest by the process described above gave no evidence of the presence of histamine. It was concluded that extraction was at least 95 per cent complete.

It is of interest to note that storage of the 5 per cent trichloracetic acid for some weeks at room temperature resulted in no loss of histamine (Table V).

Results

Values for skin histamine in relation to age are given in Table III and plotted in Graph I. The content is low in foetuses a few days before term. In connection with the estimated time it is important to notice that values obtained from the animals born to the operated mothers are markedly lower than "normal" birth values (Table IV). It is probable that the disturbance has interfered with the remaining foetuses and as a result they have been born prematurely. Thus the estimated time before birth may be too low.

The most noticeable feature of the age curve is the sharp rise starting about 14 days and reaching a double peak at 22 and 25 days, followed by an almost equally sharp drop. This peak is accompanied by an increase in the spread of values (Table III) even when calculated logarithmically (53), and consideration of the possible reasons for such a rise and fall forms the main subject of this thesis.

It was considered possible that, by suitable

analysis, the data could be divided into two populations, each with smaller values of 'σ'. Following Hatai (70) who stated that "the irregularity (in serum refractive index) shown between 18 and 30 days may therefore be due to different degrees of growth among young rats" a division according to weight seemed reasonable. But inspection of the data showed that the values were widely scattered (Graph II).

This graph shows that animals from large litters fell mostly in the range 25 - 35 g. with histamine contents 25 - 75 microg./g. and those from small litters 30 - 47 g. with histamine contents of 30 - 60 microg./g. in the age range 16 - 25 days. This is in agreement with Faulk et al. (44) who suggested that the variability of weight at a given age was connected with the size of litter. It was therefore decided to plot values for large and small litters separately. Eight or less were arbitrarily considered small litters and nine or more, large litters. The figures are given in Table VI and plotted in Graph III. Unfortunately for this purpose 8 and 9 are the commonest litter sizes and very small or very large litters relatively uncommon. However, the results seem to justify the procedure.

From these it is seen that from 20 days onwards the skin contents of animals from large litters are higher than those from small litters. Statistical calculation (Table VI) shows that the difference at

22 days is highly significant, that at 23 days significant and at 20 and 24 days there might be some difference. The spread of the two curves has unfortunately been little reduced.

Thus the rise about weaning is separated into two parts. All the animals show a rise beginning after 14 days and reaching a maximum at 25 days. Large litters have, superimposed upon this, a peak at 22 days.

After about 80 days the values become stable as indicated by the mean and the spread (σ). One result obtained indicates that individuals may remain fairly stable: a rat at 90 days gave the value 18.9 microg./g. and at 574 days the value of 22.6 microg./g. Both these values are above the average and, within the error of assay, are indistinguishable.

A few values obtained from whole rats are given in Table IV. It is seen that, although some rise undoubtedly occurs, it would not appear to be as marked as in the skin. Now skin forms approximately 15 per cent of the body (Lowrey, 100) and the average rise between 14 and 25 days is approximately 35 microg./g. in the skin. This would represent about 5 microg./g. in the total body which is of the order actually observed in the total body values (approximately 5 to 10 microg./g.). Thus if rises in other tissues occur they are either local or are balanced by simultaneous falls elsewhere.

The apparently steady rise during the first few days after birth (unlike skin histamine values which remain constant or fall slightly) may have some significance though the few figures presented here are not sufficient to establish any certain increase.

Discussion

(i) Foetal Values

Foetal values appear to be low until within a few days of birth. Three analogous observations are reported in the literature. Trethewie (162) found that guinea pig at about half-term had low histamine contents, and Feldberg and Schilf (49) showed that both ape and guinea pig foetuses had little or no histamine though the age of the foetuses are not given. Dragstedt et al. (37) found that the liver of a 200 g. dog foetus contained 5 microg./g., a value much lower than that of the adult dog (30 microg./g.). The weight of this foetus indicates that it was probably within 20 days of term (weight of pups at birth was reported as about 400 g.).

The initial rise occurring just before birth may be considered in relation to the suggested metabolic roles of histamine. Some functions in which histamine may be involved, such as gastric and salivary secretion and possibly sympathetic and sensory nerve functioning are not in operation in the foetus. Hormonal control of circulation may

occur. Feldberg and Schilf (49) state that smooth muscle in the foetus (? species) is sensitive to histamine; most functions requiring smooth muscle are not operative. The glandular mechanism is not fully functioning as Speart and his co-workers (155) showed that the foetal mouse thyroid did not take up iodine till about four days before term, and Benner (12), describing the involution of the foetal adrenal cortex, indicates our ignorance of its function before birth. In the light of Rose and Browne's (138) work this involution may cause an increase in the histamine content of tissues. A fuller consideration of foetal physiology in relation to histamine content might prove profitable. During the periods immediately before and after birth extensive adjustments to the changed environment occur, and the means whereby this change is accomplished is of great interest.

Considerations presented later in this paper suggest that part of the rise at birth may be due to a "birth stress". This will be further considered. A fall in tissue fat would tend to aggravate a rise after birth (v. sub.), but would not explain a five or six fold increase starting before birth.

(ii) Adult Values

In these experiments values from 80 days to 514 days remained fairly constant. Although old these animals were not really senile: the skin tended to show scabby patches and hair was less

dense but coarser. Neither mean nor spread showed any increase. This is in contrast with the work of Trethewie (162) who said there was an increased range of lung histamine values in older guinea pigs. His animals are in three groups approximately 55, 130 and 280 days. Feldberg and Kellaway (45) found an increased spread of values in cats' lung with increased body weight. It is conceivable that older animals than we used would show such an increase. On the other hand values may remain fairly constant until death.

Since histamine values do remain constant for a considerable period of adult life it is important to decide what are the controlling factors. Dale (31) first observed that adrenalectomised cats were much more sensitive to injected histamine than normal animals. Gottesman and Gottesman (56) reported a similar finding for rats, adding that the M.L.D. for adrenalectomised animals was a twentieth of that for normals. Rose and Browne (138) found that adrenalectomy elevated the histamine content of stomach, small intestine, liver and lung but not blood, kidney or spleen in the rat. Marshall (107) confirmed this for young (21 to 35 day) rats and included skin; the present paper also indicates that adrenalectomy may cause an increase in skin histamine.

Rose and his co-workers (79, 136) showed that the rise in lung histamine (caused partly by a

decrease in histaminase, 139) could be controlled by administering cortical hormone to the animal in doses four times greater than maintenance level. Unfortunately no experiments have been done to test whether there is a fall in tissue histamine after administering cortical hormone to normal animals. Holbrook et al. (quoted by Selye, 147) state that ACTH increases the urinary excretion of histamine.

On the other hand histamine has been shown to activate the adrenal cortex. Sayers and Sayers (143) and Nasmyth (119) showed that histamine caused a fall in the adrenal ascorbic acid content of rats, and the latter found that a release of adrenaline was not essential to the action. Work on dogs (Vogt, 165) has shown that histamine acts directly on the cortex only in high dosage and irregularly. Nasmyth (quoted by Vogt, 165) has shown that the action is via the anterior pituitary, but it is not yet known whether this is direct or through the hypothalamus. Action through the general metabolism is excluded as injected histamine acts on the pituitary within ten seconds (61).

Gotzl and Dragstedt (57) found that removal of the thyroid gland caused skin histamine to fall in the adult rat, whereas thyroid administration (10 mg. per day for 8 to 17 days) resulted in somewhat high histamine contents. There are several indications in the literature to show that a relation between thyroid and adrenals exists. Thus thyroidectomy

causes adrenal atrophy (120, 51), and hyperthyroidism results in hyper-activity of the cortex in normal rats (75) and in ovariectomised (87) and in senile (85) rats. Koelscher and Kendall (83) demonstrated that adreno-cortical hormone protects dogs against the negative nitrogen balance induced by thyroxin and that adrenalectomy caused hyperplasia and depletion of colloid in the rat thyroid. Zweimer (174) showed that thyroidectomised cats withstood the effects of adrenalectomy better than intact animals. In line with this observation may be the report that thyroidectomy and thiourea administration gave improved tolerance to anoxia in rats (see Le Blond, 91).

The inter-relation of thyroid and adrenal is probably an indirect one, via the metabolic rate of the tissues (142, 51) though the question is still open, according to Carlson (22, p. 354). Whether direct or indirect the pair of glands constitute a (hypothetical) homeostatic system: if adrenal output decreases skin histamine tends to increase but thyroid output falls causing a compensatory fall in skin histamine.

(iii) Values About Weaning

As remarked above, the sharp maximum at 20-25 days is the most striking observation in the age-curve (Graph I). The only comparable figures available in the literature are those obtained by Rocha e Silva (132). He measured the skin histamine values of the rabbit in relation to body weight

and inspection of his results indicates that the highest contents occur among the lightest animals (50-150 g.). Rabbits are generally weaned in this weight range, so that these results are consistent with the idea that a similar rise at weaning occurs in the rabbit. This is significant as the rat and the rabbit are very different in their general histamine metabolism. The rat is very resistant to histamine, whereas the rabbit is very sensitive, the histamine acting on the vascular system. If such different animals show a similar age-histamine curve in the skin the observation has a more general significance.

Marshall (107) found high and variable values for the skin histamine of rats from 3 to 5 weeks. This author was good enough to give me the individual values obtained in his experiments and these are reproduced in Table II. Unfortunately individual ages are not known. The figures were obtained 2-4 days after mock-adrenalectomy; in Section 4 it is shown that skin values of such animals probably approximates to normal values at all events 3 days after operation.

There is considerable evidence in the literature to show that many metabolites are undergoing rapid changes about the weaning period. Welsh and Hyde (168) showed a sharp maximum for the content of acetylcholine in the brain stem and medulla but not the pallium or cerebellum of the rat. Sinclair (152)

found that the percentage of phospholipid fatty acids in the whole rat was maximal at 35 g. (about 22 days) when measured on a fresh tissue basis; on dry weight a gradual fall from birth was noted. Cole and Koch (26) found the acid-insoluble phosphorus in the rat gastrocnemius muscle was maximal at weaning, and this was confirmed by Horvath (74). Lowry and Hastings (101, p. 123) recalculate the latter's data, however, for muscle fibre content and show that a stasis occurs about weaning in a general decline.

Enzyme systems may also show such effects, as was demonstrated by Tyler and von Harreveld (163); these authors found that the oxygen uptake of rat's brain was maximal 28 to 49 days. Faulk et al. (44) found great irregularities of lipase activity about weaning and, although the results are not so clear, protease activity seemed to show an increase just after weaning. Values reported by Hatai (68) indicate that total body protein may be at a minimum at weaning. Toyama (161) found serum albumin maximal at 30 days in the rat. His results are not clearly related to those of Hatai (70) who measured the refractive index of rat serum and found minimal values at about 23 days.

Figures given by Benedict and MacLeod (11) indicate that heat production (an index of basic metabolic rate) may reach a maximum about 20 days when measured at 28.9°C. (but not when measured at 25.7°C.). These results are supported by those of

Mitchell and Carman (112) and may be related to the important observation of Monroe and Turner (115) that thyroid secretion is higher in rats just after weaning than in older animals. The minimum reaction time to an electric shock in 21 day old rats by Brody (18) may also be related to these observations. Bodansky and Duff (15) found 22 to 36 day old rats more tolerant to administered thyroxine, and Belasco and Menkin (10) obtained similar results at about 60 days compared with animals at about 500 days.

Age changes in rat skin have also been described. Andrews (6) finds rat skin markedly different at 21 days from adult samples on the basis of histological observation. The dermis is much more cellular in 21 day samples; the epidermis (stratum germinativum) shows prominent nuclei with little surrounding cytoplasm, and is much thicker on the abdomen than at about 300 days. Thicknesses on the back are, however, similar. He suggests that these differences should be kept in mind when using skin of this age as experimental material.

The work of Bruckman and Zondek (19) and Schwarz (146) on non-haemin iron in human liver and of Zorzoli (173) on acid and alkaline phosphertases in mouse liver, show that the phenomenon is not confined to the rat. Barlow et al. (8) found that the most marked changes of water and chloride content of chicken tissue occurred in the first month.

On the other hand, work has shown only slight

variation in the rate of change of total magnesium and water (Greenberg and Tufts, 62), total solids, creatine, creatine phosphate, hexose phosphate and acid soluble phosphate (Horvath, 74), in non-protein nitrogen in the brain (Hatai, 69) and total chloride (Winter, 171) in the rat. Weaning appears to have no influence on the total phosphorus content (Sherman and Quinn, 148) on the vitamin A content of the liver (Guerant, 63), or on the total carbohydrate metabolism (Reiner, 127) of the rat, nor on the muscle, liver or kidney acid soluble phosphorus of human beings (Pincussen et al., 126). In some of these cases, however, the age range of the groups may have been sufficiently large to obscure such effects.

Various suggestions have been made as to the reasons for such changes. Cole and Koch (26) seek to explain their findings on the gastrocnemius muscle in the fact that the hind legs are little used before weaning. Horvath (74) has suggested that the high adenosine tri-phosphate content of young animals acts as a reserve against muscle fatigue. Britton and Kline (17) state "the lower resistance of 20-40 day old rats (to anoxia) may be referable to the marked speeding up of bodily activities on their becoming independent at weaning", and Asdell (7) adds to this the possible importance of taking solid foods.

Hammeth and Tokuda (65) measured the effects

of extracts of rat thyroid on isolated segments of rat duodenum and pointed out that "the jogs in the curve at weaning and puberty..... point strongly to the probability that these two periods..... are critical points in development and points deserving of special investigation". The uncertainty attached to the nature of the substance or substances measured by these authors does not detract from the value of their remark: Chanutin (23), on the basis of estimations of creatin, total nitrogen, ash, body solids and ether solubles in whole rats, remarks, "The critical period in the chemical growth of rats occurs about the time of weaning. It is quite possible that rats at this age are undergoing a metabolic as well as a "chemical"crisis". The present paper confirms this suggestion.

The possible reasons for the peak observed in skin histamine can be conveniently discussed under three headings, namely, mechanical (that is, variation in unrelated skin elements causing an apparent change in histamine content), dietetic and systemic.

(i) Mechanical Causes

Since the epidermis may be richer in histamine than the dermis (p. 13, para 2) a relative change in the proportions of these elements would result in an apparent change in total histamine content. Figures are not available in the literature, but it would seem possible that the epidermis undergoes thickening in the period about weaning; a subsequent rapid

decrease appears less likely, though Andrews (6) finds epidermal thicknesses of 38 and 22 microns at 21 and 300 days respectively. Rocha e Silva's (132) results show no clear relationship between thickness of the whole rabbit skin and histamine content; indeed thickness is minimal when histamine is maximal and at a maximum shortly after, when the histamine content is falling. Subsequently they decline in parallel.

Thus the rise between 16 and 25 days may be caused in part by an increase in epidermal thickness but the subsequent fall is probably a result of other factors. Further it is conceivable that a sharp change in the water or fat content of the tissue could cause the observed effect. The water content of the skin falls during the pre-weaning period from about 77 per cent at 7 days to 59 per cent at 20 days. It appears to rise slightly after this to 63 per cent at 42 days (Lowrey, 100), Winter, 171). The dry-weight values of histamine for these days are 28.4, 71.0 and 44.4 microg./g. respectively, so that the rise would be only slightly reduced on this basis. (This calculation assumes that histamine is largely combined with the "solids" and is not in solution to any sensible extent.)

As histamine is insoluble in fat, adipose tissue would simply act as a diluent when the whole tissue is assayed. Thus any depletion of fat reserves would cause an apparent rise in skin hista-

mine. The figures of Adams (2) for the fat content of rat skin are somewhat sparse over the range required but he gives 20.7 per cent at 10 days and 10.6 per cent at 36 days. These together with Lowrey's (100) data for skin dry weight (including fat) are consistent with the idea that fat rises during the pre-weaning period and falls subsequently. As Hatai (68) reports that rat milk is rich in fat, such a rise is to be expected; concerning the fall, Chanutin (23) states, "The increased activity and metabolism, together with the change of diet at weaning, must account for much of the decrease in fat content".

Both Hatai (68) and Chanutin (23) report a rise in total body fat (though Hatai's figures are somewhat higher than Chanutin's) up till 20-23 days, followed by a fall. Unfortunately both authors permitted the young to remain with the mother and wean themselves, whereas in our experiments the mother was removed at 21 days. If it is assumed that the increase in skin fat is proportional to that of total body fat between 7 and 20 days, then the skin content may be as high as 36 per cent at 20 days. Should this fall to 10 per cent in five days then the average histamine contents at these two days on a fat-free basis are about 58 microg./g., that is, there is no change. The author does not consider this likely as Lowrey (100) reports that the skin dry substance, including fat, is only 41

per cent at 20 days, and on an approximate estimate from Adams' (2) figures the non-fat dry substance amounts to at least 15 per cent.

There are other objections to this explanation. The skin histamine content increases from 16 days onwards, though no evidence suggests that skin fat starts to fall at this time: the above calculation is invalid if that were so. Further the fall in histamine starts at 25 days, whereas there is no indication that the decrease in fat has been arrested at this time, still less that an increase has started.

It cannot be denied that the changes in fat content probably contribute to the observed histamine content changes. However, the extent of the changes and their time relationships indicate that this cannot be the only, or even the major, factor involved.

(ii) Diet

The second possibility is that the change of diet affects the skin histamine. This may be due to an increase of histamine or histamine precursors in the food, to a change in the intestinal flora or to an alteration in the internal metabolism generally as a result of the change of diet. This last is indistinguishable from causes described in section (c) below.

Hatai (68), who permitted animals to wean themselves, concluded that solid food was first taken at

17-18 days and weaning was complete at 23 days. The figures he gives for the refractive index of serum are consistent with this idea. The present writer has seen rats nibbling at rat cake as early as 14 days, though whether any was eaten remains in doubt. However, the actual times of starting solid food and of natural weaning probably depend on weight as well as age.

Some information on the histamine content of the diet was obtained. Milk (cows'), bread and rat cake were investigated. In the case of milk and bread, extraction by the normal procedure of Code resulted in a solution which relaxed the gut or caused a contraction not inhibited by mepyramine solution. Histamine added to the final solution was sometimes inhibited. It was found, however, that extraction of the alcohol-dried residue with alcohol separated the interfering substance(s), and the contents were found to be 0.1 microg./g. in the case of bread (4 samples) and 0.5 microg./ml. in the case of milk (2 samples). 6 microg. of histamine was recovered to the extent of 58 and 75 per cent in two experiments by this technique. The figures obtained by Toni (160)(0.25 - 1.0 microg./ml., average 0.57 microg./ml.) and Rex-Kiss and Went (130) (0.05 - 1.0 microg./ml., no average given) for human milk accord well with these. It is assumed that rat's milk would contain an equally low concentration though this may not be so. Rat urine has a high

histamine content (Adam, 1).

Rat cake varied in content between 0.8 microg./g. and 12.8 microg./g. (Average of 18 samples 5.5 microg./g.) From the rat cake content and assuming the maternal milk to contain negligible amounts of histamine, the minimum quantity of rat cake that would have to be consumed to give the observed rise may be calculated as follows. From Lowrey's (100) figures for 7 and 20 days the weight of the skin at 16 days would be about 4.4 g. The total histamine contents are therefore 120 microg. and 186 microg. at 16 and 20 days respectively and the skin content has risen 15 microg. per day. Thus the animals would have to eat at least 2 g. of rat cake a day even if all the histamine entered the skin. This seems highly unlikely and the consumption of even larger quantities of rat cake at such an age equally improbable. As was shown above (page 31, para. 3), from 20 days onwards the animals with the highest skin histamine values are those who would be expected to eat less rat cake. One must conclude that no significant part of the observed rise can be due to ingested histamine.

That part of the rise (starting about 16 days and reaching a peak at 25 days) is due to intestinal flora is more probable. Wilson (170), working with adult rats, has shown that chloromycetin reduces the urinary output of histamine at a time when it is known to have reduced bacterial population of the

intestine. This indicates (though it does not prove) that such organisms are a source of histamine for the rat. Unfortunately no data for the intestinal flora of the suckling rat are available.

(iii) Systemic

Although changes in epidermal thickness, fat content or intestinal flora may account for the slow rise, there still remains the superimposed peak in the larger litters. The time relations point to weaning as a probable cause. It may be that, in the case of the small litters, the food obtained in the first 21 days enables the animals to reach a stage in development where weaning is possible; with large litters this is not so. Such a suggestion is consistent with the idea that development is better measured (in the rat at all events) by weight rather than chronological age, an idea supported by the work on survival on sub-optimal diets (103, 104).

As all animals were removed from the mother at 21 days the members of large litters must have undergone stress by starvation to some extent before they became fully accustomed to meeting their whole dietary requirements from the food provided. Confirmation of such an hypothesis might be sought by not weaning a large litter or by weaning a small litter early. The results of such experiments are given in Tables VII and VIII.

It is clear that not weaning a large litter

does prevent skin histamine rising to the high values observed just after 21 day weaning in such litters. A rise to 26 days still seems to occur but the figures at 22 days are comparable with those of 21 and 23 days.

Not weaning a small litter sometimes (Table VII, Col.1) caused even lower values to be obtained. Together with the very poor correlation of skin histamine with weight, even allowing for litter size 9Graph II), it was considered possible that the removal of the mother may have had other effects than nutritional. The young may miss the warmth of the maternal body and the occurrence of a purely "psychological" shock cannot be altogether excluded. The arbitrary division of litters into large and small may have caused some animals to be allotted to the wrong group.

It is seen (Graph V) that the average curve of all unweaned animals resembles the curve for small litters. The resemblance would be greater if values less than 30 microg./g. were omitted. However, five such values were obtained in weaned animals between 20 and 24 days. Of these four belonged to small litters and the fifth was slightly above average weight for a large litter, being 29 g. at 20 days (see Graph IV).

Unfortunately in only one experiment was the effect of early weaning investigated. (In this, as in other parts of the work, two difficulties were

faced. Litters of very small or very large size are rare and in small litters there is less experimental material.) A litter of 5 animals was weaned at 16 days, when their weight (about 30 g.) was slightly higher than the average weight (27.5 g., Graph IV) of a large litter member at 21 days. Values of age, weight and skin values are given in Table VIII. It is seen that a sharp rise during the subsequent two days occurs with a concurrent fall in body weight. Now the rise after 21 day weaning occurs within a day so that the time relations are not entirely the same. This point is discussed below (Section 4 (ii)). Nevertheless, the result indicates that premature weaning does affect skin histamine.

If, then, the rise between 21 and 23 days in large litters is due to the removal of the mother, why does such a procedure cause a rise? In Section 4 evidence is presented to show that a rise in skin histamine occurs under stress and the relationship between this finding and the above data is discussed.

Another factor which may cause a rise is the hyper-activity of the thyroid (115). The adrenal would also appear to be more active than normal (76) but if the increase is less than that of the thyroid (that is, the tissues are hypocortical) then histamine contents might be expected to rise. Much further work is required to elucidate this point.

4. EFFECT OF STRESS ON SKIN HISTAMINE

Methods

Skin was sampled as above (p. 26). The wound was closed with Michel clips and the animal subject to mock or actual bilateral adrenalectomy through a dorsal mid-line incision. The operation took 3-10 minutes (generally about 6 minutes). The animals were returned to their cages. After a variable interval of time a second sample of skin was removed from the opposite flank.

The two samples were assayed by the technique described above (p. 27, Section 3(ii).

Results

Three age groups were investigated for the effect of removing the skin sample plus mock adrenalectomy (referred to as normal groups). The results are presented in Tables IX, X and XI and in Graph VI.

The interpretation of the figures for the youngest group is rendered difficult by the large variation in values over one to three days. The values in Table IX are corrected from Graph III and Table VI, and Graph VII shows the effect of this correction.

The general reaction in all three normal groups is the same. After some possible fluctuation the values rise to a peak at about 24 hours and then fall during the next day or two. In the oldest group the peak occurs between 12 and 24 hours and the return to normal has taken place by 48 hours.

The 50 day old group seem to have their maximum between 24 and 48 hours and have returned to normal by 72 hours. The youngest group resembles the oldest in returning to normal by 48 hours; in view of the few results, the fact that the animals are already under stress and the derived nature of the data, it is considered that these results are not trustworthy but are included to indicate a probable similarity.

In the case of the 50 day old group, the early change tends to be related to the initial value; animals with high initial skin histamine tend to show a fall, those with low values a rise (Table X and Graph VIII). Subsequent changes are similar so that the two curves are parallel and separated by about 20 per cent, except at 72 hours where the difference is greater. Such an effect was not observed in the oldest group of animals, and this is taken as evidence that the effect in the 50 day old group was probably not due to errors in estimating the initial value. The youngest group does not appear to show such a separation but the number of values may be too small.

The two younger groups were paralleled by adrenalectomy experiments (Tables XII and XIII and Graph IX). Again the 22 day old figures must be corrected and the effect of this correction is shown in Graph X. The initial parts of the curves show no difference from the normal curves; this

may be due to the fact that circulating hormone is sufficient for a few hours (Gilman and Goldberg, 55; Love, 99).

Unlike the normal curves, however, the adrenalectomy curves do not appear to return to initial values at a time (48 and 72 hours for 22 and 50 day old groups respectively) when the normal values have so returned. However, comparison of the means at 72 hours for normal and adrenalectomised animals (50 days) by Student, 't' indicates there is no significant difference. Owing to the small size of the groups the evidence that skin histamine remains high is not conclusive. In both groups the peak at 24 hours seems higher than that of the corresponding normal curves. The peak for the 22 day old rats is lower than that of the 50 day old animals, possibly because of the stress the younger animals are already undergoing owing to weaning.

The results of such experiments cannot be properly evaluated without information on the variation in content of samples taken simultaneously from both flanks of the same animal. Table XIV shows the results of such an experiment in which samples were taken within three minutes of one another. The percentage differences have been compared with those in the "stress" experiments and values for Student's 't' are incorporated in Tables IX - XIII. Inspection of the values given in Table XIV show that the second sample is randomly higher or lower

than the first. However, the possibility cannot be excluded that amongst the younger animals a small effect was produced by anaesthesia or injury upon the skin histamine values of the second sample, even within this short time. Yoffey and Baxter (172) indicate that anaesthesia may alter the histochemical appearance of the adrenal glands.

Discussion

(i) The Form of the "Stress" Curve

It is clear from the literature and from the adrenalectomy experiments performed that the adrenal gland is involved in the reaction. Rose and Browne (138) have shown that adrenalectomy results in increased histamine content of several rat tissues for up to 12 days, and both Marshall's (107) results and those of the present paper suggest that skin may be added to their list. The decreased ability of adrenalectomised rats to inactivate histamine is a probable cause (137).

It is not known (Sayers, 142) whether tissues not directly subject to stress have increased requirements of cortical hormone, though stressed tissues certainly need more. Initially the increased output of the adrenal will satisfy these requirements. This corresponds to the "shock phase" of Selye (147). When the effect of stress begins to diminish, cortical output will fall towards normal.

Now the work of Rose (136) indicates that an

increased quantity of histamine requires an increased amount of hormone to reduce it; levels of cortical hormone administered to adrenalectomised rats to reduce tissue histamine to normal were higher than the maintenance dose. Therefore, if histamine is still being produced 12 to 24 hours after injury the tissues will be in relative hypocortism at all events in respect of their histamine metabolism. The fall in skin histamine is due to a redress of this imbalance probably due to a second increase in adrenal output ("secondary shock"). It is well-known that exogenous (32, 119, 142, 143) and endogenously released (105, 123) histamine can act as a stressing agent. A reduction in histamine production is not excluded from playing some part in the observed fall.

This hypothesis is well supported by the histochemical findings of Yoffey and Baxter (172). They show a depletion of fatty material in the zona reticularis at up to 6 hours, a return to normal at 12 hours and further depletion at 24 and in some cases 48 hours after administering adreno-cortical trophic hormone to adult rats. It is of interest to note that they find the time relationships of individual animals somewhat variable; this may account for the rather wide scatter of values reported here.

Perhaps the most important fact emerging from this work is that after injury non-affected portions

of skin show a change in histamine content. This is confirmed by an analysis of the figures published by Kawaguchi (80) for guinea pigs, by Tarras-Wahlberg (159) for rabbits and cats, and by Dekanski (34) for cats, though Loos (98) and Kisima (quoted by Dekanski, 33) did not observe such effects in the rabbit or in the dog respectively. The possibility of these changes should be borne in mind when unaffected areas are used as controls in this type of experiment.

(ii) The Age Change of the "Stress" Curve

This change is slight. As stated above (p. 51, last para.) the curve for the youngest group cannot be considered comparable with the two older groups as the animals are already under stress as judged by skin histamine changes. It is perhaps surprising that such a curve has been obtained at all for the youngest group and indicates the need for further experiments on successive stresses.

The rise and fall observed occur earlier in the 330 day group than in the 50 day group. As the older animals were considerably bigger the injuries may have been relatively less; the figures (Tables X and XI) for percentage injury suggest this but may not fully express the possible differences as they do not include the mock adrenalectomy. One of the fundamental difficulties in this type of experiment is to administer the same relative degree of stress to animals of different size.

The literature indicates that reaction to stress varies with age. Britton and Kline (17) showed that though resistance to anoxia was high at birth (using air at 160 mm. Hg.) it was at a minimum at 30-40 days. Adolph (3) reports a minimum value in his series (exposure to nitrogen and cold) at 17-21 days. He states that this age group gives the same value as adult animals but reports no figures for higher ages. Grad et al. (58) administered nembutal and ACTH and found a significantly greater response in 50 day old rats than in 240 day old rats as judged by the fall in circulating eosinophils.

Handler and Follis (66) found 50 g. (about 30 day) rats less resistant to cystine and choline than 150 g. (about 60 day) and 300 g. (120-300 day) animals. 42 day-old rats are more resistant to histamine after adrenalectomy than adult animals, according to Perla and Gottesman (125). Chen and Robbins (24) showed that whereas animals of about 28 days were less sensitive to morphine, sulphapyridine and a barbituate, they were more sensitive to ephidrine than adult animals. Freedman and Himworth (52) find a maximum resistance to DIP in rats 18 days old. However, as resistance is at a minimum at 32 days and no figures between these two ages are given, the possibility of a sharp fall immediately after weaning is not excluded. The weights of the animals used are unfortunately not given.

Both Cowie (29) and Sisson and March (153) demonstrated that animals at weaning have a shorter survival time than older animals after adrenalectomy. Gilman and Goldberg (55), however, record no such minimum. The results of this type of work depend very much on technique and, as indicated above, there will be a tendency to inflict a relatively larger wound on the younger animals. In all three papers it is the youngest group that has the shortest survival time. Gilman and Goldberg's figures indicate that survival period may even be at a maximum at weaning. This may be related to Jackson's (76) observation on the size of adrenal cortex. His figures seem to indicate a maximum size though Mitchell (111) found no increase in the mitotic figures of the adrenal gland about 21 days.

Although these results appear somewhat conflicting they are all in accord that the rat is in an abnormal metabolic state at and just after weaning and that adrenal cortical and thyroid outputs are probably high. The review of the literature given above (pp. 38-42) also suggests a functional disturbance at this age. Jackson's figures show that the adrenal gland is still relatively large at 56 days; thus the greater delay in the rise of skin histamine at 50 days compared with the adult may be caused by a greater output of cortical hormone in the younger animals. Here the one result of early weaning may be recalled (Table VIII). The rise

occurred over a period of two days and it is not known whether the maximum had then been reached. This is consonant with a larger hormone secretion. However, without further data on the thyroid involvement, such a simple picture can only be a first approximation to explain the observed changes. The marked fall in body weight shows that these animals are not entirely analogous to members of large litters weaned at 21 days.

Mention may be made, finally, of the possible difference in behaviour between animals with high and with low histamine content in the 50 day old group (Graph VIII). The results are too few to draw any definite conclusions. It is conceivable that animals at this age might show significant difference depending on their previous stress experience; in that case, possibly those with low histamine content have had less stress than those with high, and the administered stress tended to move the histamine content towards the higher level. It would appear that this effect (if it occurs) is superimposed on the general fall in skin histamine and ultimately reaches an end-point beyond which response to stress causes no permanent change in the skin histamine content. No correlation between litter size and skin histamine content at 50 days was observed.

(iii) The Relation of the "Stress" Curve to Changes at Weaning

The difference between small and large litters has already been shown to be consequent upon weaning. In Graph XI the differences between the small litter values and the large litter values between 21 and 24 days are expressed as a percentage of the small litter values (Table VI). For comparison the normal curve for 50 day old rats is also plotted. The resemblance of the two curves is strong enough to suggest that the effect is due to a stress on the organism when weaned prematurely. The difference between small and large litters at 21 days may be due to the fact that samples were taken up to 6 hours after weaning.

This striking similarity may be due to one of two causes. The two stresses may have been approximately equal, in which case it must be accepted as a piece of good fortune, or the response is not related to the magnitude of the stress. There is little evidence of any correlation between size of injury and histamine change in the results for stress (Tables X and XI) although as only part of the injury was measured such a correlation may have escaped observation. If the response is unaffected by the degree of stress, the difference observed between the age groups is more certainly due to a change in reactivity of the organism as the question of administering different degrees of stress will not arise.

Without further experimental work it is impossible to decide between these two possibilities. The fact of the similarity still remains, however. It is therefore considered probable that the observed difference between small and large litters just after weaning is due to the stress undergone by the lighter animals after removal from the mother.

The possibility of stress also arises at birth. The available figures are few but indicate a rise during the first 6 hours, namely, 1 hour 23.3, 3 hours 23.6, 26.1, 6 hours 35.8, 29.2 microg./g. Values have returned to "normal" at 2 days. However, in the light of Benner's (12) work on the involution of the foetal adrenal, this point must await further elucidation.

5. CONCLUSIONS

The age curve of histamine in the rat skin exhibits a general, fairly constant level of 10 to 20 microg./g. up to 500 days. There are, however, two exceptions, the peak about birth and the peak about weaning. Between these two disturbances values not much above the normal adult figures are obtained. The rise at weaning has been shown to be separable into two parts. Observations on the histamine content of bread and milk (less than 0.5 microg./g.) and of rat cake (about 5 microg./g.) show that these cannot account for the steady rise observed between 16 and 24 days. This may be due to alterations in the intestinal flora on changing from a milk diet to solid food or to hormonal imbalance. These causes probably account, in large measure, for the other irregularities noted in the literature at about this age. Changes in epidermal thickness and tissue fat content may influence the change but are not of prime importance.

The peak observed at 22 days in large but not in small litters is ascribed to the shock of premature weaning. Not weaning a large litter eliminated this peak and the suggestion is further supported by weaning a small litter prematurely and by the results obtained after traumatic stress. These results indicate that the increase in skin histamine after such treatment may be caused by a

relative hypocortism.

Apart from this theoretical aspect the results reported here indicate that careful consideration should be given to weaning schedules to ensure that all litters are, as far as possible, similar. The practice of some authors of allowing the litter to remain with the mother till thirty days has much to recommend it.

The rise before birth may again be related to changes in the thyroid and adrenal glands and forms part of the general metabolic changes at parturition.

An important point arising from Section 4. is that skin not affected directly by the stressing agent did show a change in histamine content. The use of unaffected areas as simultaneous controls should clearly be used with caution.

It would appear that, in general, changes in the histamine content of the skin indicate a disturbance of the hormonal balance of the adrenal and thyroid. These changes may also be an indicator of disturbed protein metabolism (133, 134). The gerontological importance of such disturbances has been pointed out in the Introduction, and theory suggests that work on successive stresses might be informative. The results indicate that animals about 50 days old might show differences in their skin histamine reactions which could be related to their previous experience. Such differences do not appear to exist in adult (330 day) animals.

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Tables

Table I

VALUES FOR SKIN HISTAMINE REPORTED IN THE LITERATURE

(Given as Histamine Base)

1	2	3	4	5	6
Species	Site	Mean Value (microg./g.)	Range (microg./g.)	No. of Observations	Reference
Man	Breast	6.3	5.7 - 7.6	9	67
	Thigh	8.4	8 - 8.8	3	67
	-	19.8 *	17.3 - 23.7	14	124
	Arm ?	10.5	5 - 24	33	121
	Arm ?	5.1, 4.6	-	2	59
Rat	-	15.7 *	12.5 - 20.6	4	108
	-	11.5 * ?	10.9 - 16.5	10	57
	Back	12.6	10.2 - 15.6	14	64
	Abdomen	26.4	10.9 - 37.5	7	40
	Abdomen	34 *	-	unknown	50
Rabbit	-	3.7	2.8 - 5.6	5	131
	Abdomen	5.5 /	3.9 - 6.7	4	159
	Abdomen	2.9	0.4 - 6.4	8	132
	Abdomen	6.3, 8.8	-	2	59
Cat	Flank	12.0 ϕ	12.0 - 12.1	3	67
	Abdomen	20.2 /	11.2 - 32.1	4	159
	Abdomen	18.8, 13.0	-	2	59
	Leg	32 *	-	1	105
	Leg	21.7 *	13.5 - 25.0	10	47
Guinea pig	Abdomen	7.6	2.5 - 12.5	9	40
	Abdomen	5.0	2.7 - 6.4	5	59
	-	2.9	-	9	114
Mouse	Whole skin	16.7 o	15.3 - 18.1	4	33
		24.4	-	4	33
Dog	Hind leg	8.3 *	4 - 23.5	15	48

* Original values assumed to be given as base.

/ Original values assumed to be given as di-hydrochloride on the basis of a previous paper (158). Values obtained 5 minutes after ultra-violet irradiation.

ϕ Values obtained 1 hour after burning opposite flank.

o Histamine extracted by electrodialysis.

Table II

Individual values obtained by Marshall
after mock adrenalectomy

Time after operation (days)	Values (microg./g. fresh tissue)	Average
2	63.5, 68.8, 49.0, 68.8, 57.1, 39.7, 60.5, 46.9	56.8
3	34.0, 66.5, 67.3, 43.1, 42.6, 63.2, 47.0, 42.3	50.8
4	43.6	(43.6)
	Average 53.2	

Table III. SKIN HISTAMINE VALUES AT VARIOUS AGES

Age (days)	Mean Value (microg./g.)		No. of Animals	Age (days)	Mean Value (microg./g.)		No. of Animals	Age (days)	Mean Value (microg./g.)		No. of Animals
4* Before birth	4	-	(1)*	25	56.5	12.24	14	93	16.8	2.82	3
2* Before birth	7.7, 8.1	-	(2)*	28	45.7	9.74	5	133	17.5	(w=4.8)	2
0	28.8	5.45	10	31	31.9	6.48	6	153	15.4	2.41	4
2	18.2	3.30	6	44	27.9	5.59	49	158	16.1	2.32	5
5	21.9	3.27	3	45	27.1	8.14	18	235	15.8	2.29	5
8	21.9	1.38	4	46	23.6	8.05	9	286	15.5	1.92	5
10	18.1	2.22	4	47	27.3	6.43	16	317 - 326	18.9	2.20	15
14	22.4	2.87	3	49	26.1	5.38	10	326 - 333	17.3	5.83	21
16	26.9	2.79	5	50	27.2	5.47	6	334 - 339	12.8	4.47	10
18	33.0	3.98	4	53	24.3	3.45	10	341	16.4	2.10	10
20	41.9	8.87	14	55	26.7	2.13	5	345	12.1	3.59	6
21	39.1	7.34	16	65	20.7	2.59	4	354	15.1	2.46	4
22	56.4	11.89	21	70	18.6	2.28	7	460	15.4	3.07	4
23	47.9	10.92	21	80	13.9	1.81	5	507 - 514	17.9	3.22	9
24	55.1	13.46	20								

* See text (Section 3 ii)

Table IV

Birth values of animals born to mothers
who had foetuses removed 2 or 4 days earlier
compared with "normal" values

"Normal" birth values	33.4, 25.2, 27.2, 25.2, 39.0, 35.8, 29.2, 23.2, 26.1, 23.6	Average 28.8	Student's 't' test Difference 5 - 10% Significant
"Operated" values	19.4, 23.2, 23.8, 18.5, 19.1	20.8	

Table V

TOTAL HISTAMINE CONTENT OF ANIMALS AT VARIOUS AGES

Age (Days)	Weight (g.)	Content (microg./g.)
½	5.5	4.5
2	8	7.5*
4	11	9.5
5	10	11.7
7	14	8.7
20	35	17.7
22	39	11.2 /
44	109	13.1
52	158	7.0
70	220	6.1
70	214	5.7
128	178	9.4
320	349	4.1
320	350	4.2
321	215	5.7
321	189	4.1

* 8 microg./g. 6 weeks later

/ 11.2 microg./g. 8 weeks later

Table VI

Skin histamine of small and large litters compared : 0 - 31 days

	Small litters				Large litters				
Age (days)	No.	Average Weight (g.)	Average Content (microg./g.)	σ	No.	Average Weight (g.)	Average Content (microg./g.)	σ	Student's 't' test
0	1	(5)	(23.2)	-	9	4.8	29.4	5.4	
2	2	7.5	19.7	(w = 8.6)	4	6.3	17.5	1.9	
5	3	9	21.9	3.3	-	-	-	-	
8	4	11.8	21.9	1.4	-	-	-	-	
10	4	20.3	18.1	2.2	-	-	-	-	
14	3	23.5	22.4	2.9	-	-	-	-	
16	1	30	27.4	-	4	25	26.8	2.2	
18	-	-	-	-	4	27	33.0	4.0	
20	7	32.1	36.9	3.2	7	27.3	46.9	10.2	2 - 5%
21	9	39.0	36.6	7.9	7	27.8	42.2	5.5	> 10%
22	7	35.0	42.9	6.8	14	28.8	63.2	7.0	$\ll$ 0.1%
23	8	37.8	41.4	8.5	13	27.7	51.9	9.2	1 - 2%
24	17	39.5	51.9	5.8	7	34.5	61.5	20.4	5 - 10%
25	4	40.4	48.1	5.4	11	34.5	57.6	14.2	> 10%
28	-	-	-	-	5	33.8	45.7	9.7	-
31	3	50.7	28.5	1.72	3	(59.7)	35.3	8.3	> 10%

Table VII

Skin Histamine Values of Non-Weaned Litters (microg./g.)

Litter Size	8	4	10	9	No.	Average	σ
Age (days)							
20	40.7		29.7, 41.5		3	37.3	6.6
21	39.5		33.9	44.6, 38.9	4	39.2	4.4
22	27.6	43.5, 28.5	46.1, 52.1	43.5, 42.5	7	41.0	8.4
23	20.8		46.5		2	33.7	-
24	29.7		50.7	45.3, 29.0	4	38.7	11.0
25	25.6		39.7, 38.8	43.8, 28.1	5	35.2	7.9
26		44.3, 50.1	53.2	32.3	4	45.0	9.2

Table VIII

The Effect of Early Weaning

Litter of 5 weaned at 16 days

Age (days)	Weight (g.)	Value (microg./g.)	Average (microg./g.)	Increase (%)
16	30	27.4		
17	27 26	32.4, 32.4	32.4	18%
18	24 24	43.9, 46.8	45.4	66%

Table IX

Skin histamine values of 22-25 day old rats before and after remote injury

1	2	3	4	5	6	7	8	9
Period (Hours)	Initial Value (microg./g.)	Final Value (microg./g.)	Age (days) at start of experiment	Litter Size	Average Normal Change (microg./g.) (Graph III)	Percentage Change Col 2-Col 3 (uncorr.)	Percentage Change Col 2-Col 3 (corr.)	Statistical Data *
6	46.4	47.2	23	5	+ 2.6	+ 1.7	- 3.9	
	51.2	54.6	22	2	- 0.5	+ 6.6	+ 7.6	σ_u = 19.4
	40.4	37.4	23	2	+ 2.6	- 7.4	-13.8	σ_c = 13.7
	34.1	48.8	21	6 - 3	+ 1.6	+ 43.1	+38.4	t_a = 10%
	40.0	46.6	23	5	+ 2.6	+ 16.5	+10.0	t_b = 10%
Average						12.1	7.7	
12	44.1	49.7	25	6 - 8	- 2.0	+ 12.7	+ 17.3	
	45.9	42.8	25	6 - 8	- 2.0	- 6.8	- 2.4	
Average						+ 3.0	+ 7.5	
24	66.0	52.6	22	12	- 11.4	- 20.3	- 3.0	σ_u = 14.0
	70.4	76.0	22	12	- 11.4	+ 7.7	+ 24.2	σ_c = 14.9
	57.3	65.4	22	13	- 11.4	+ 14.1	+ 34.0	t_a = 5-10%
	58.1	57.6	22	13	- 11.4	- 0.9	+ 18.8	t_b = ~10%
	68.3	60.2	22	13	- 11.4	- 11.9	+ 4.8	
Average						- 2.3	+ 15.8	

Table IX contd.

1	2	3	4	5	6	7	8	9*
48	29.1	44.2	23	12	+ 5.8	+ 52.0	+ 32.0	σ_u = 32.0
	56.6	63.9	23	12	+ 5.8	+ 12.9	+ 2.7	σ_c = 19.3
	65.2	33.1	23	12	+ 5.8	- 49.2	- 58.0	t_a $\gg$ 10%
	75.6	71.6	25	9 - 10	- 8.6	- 5.3	+ 6.1	
	86.5	64.4	24	8	- 2.0	- 25.6	- 23.2	t_b = $\gg$10%
	70.9	73.1	22	Large	- 8.0	+ 3.1	+ 14.4	
	49.4	40.6	21	Large	+ 9.2	- 17.8	- 36.4	
Average						- 4.3	- 8.9	

σ_u = standard deviation of the uncorrected percentages

σ_c = " " " " corrected "

t_a = Student's 't' between corrected and uncorrected values

t_b = " " " " values and values given in Table XIV

Table X

Skin Histamine Values of 50 day old Rats
Before and After Remote Injury

1	2	3	4	5	6	7	8	9
Time after operation (hrs.)	Sex	Body wt. (g.)	Wt. of sample (mg.)	Percentage Injury/	Initial value (microg./g.)	Final value (microg./g.)	Percentage Change	Statistical Data
1/3	F	120	363	6.30	37	32	- 13.5	
	F	109	367	0.34	23*	25	+ 8.7	σ = 15.2%
	F	111	326	0.29	28	24	- 14.3	t $\gg$ 10%
	M	150	581	0.39	23*	23	0	
	M	135	692	0.51	23*	28	+ 21.7	
	M	127	582	0.46	25	21	- 16.0	
Average		125.3	485.2	0.32	26.5	25.5	- 2.2	
1	M	119	912	0.77	32	24	- 25.0	
	M	112	587	0.52	20*	21	+ 5.0	
	M	104	569	0.55	9*	13	+ 44.4	
	M	105	642	0.61	18*	25	+ 38.9	σ = 27.7%
	F	107	558	0.52	26	30	+ 15.4	t $\gg$ 10%
	F	114	644	0.56	24*	26	+ 8.3	
	F	111	508	0.46	26	26	0	
	F	114	490	0.43	32	38	+ 18.8	
	M	171	573	0.33	24*	27	+ 12.5	
	M	173	519	0.30	27	22	- 18.5	
	M	104	467	0.45	26	46	+ 76.9	
	M	135	542	0.40	28	30	+ 7.1	
Average		113.7	584.3	0.49	24.3	27.3	+ 15.3	
3	M	123	277	0.22	24*	24	0	
	F	101	552	0.55	29	29	0	
	M	115	498	0.43	21*	13	- 38.1	
	M	123	448	0.36	21*	16	- 23.8	
	F	103	282	0.27	30	21	- 30	
	M	142	271	0.19	25*	25	0	σ = 29.8%
	M	144	239	0.17	32	19	- 40.6	
	M	146	248	0.17	29	26	- 10.4	t $\gg$ 10%
	M	133	290	0.22	18*	20	+ 11.1	
	F	111	357	0.32	19*	29	+ 52.6	
	F	102	336	0.33	27	31	+ 14.8	
	F	100	306	0.31	24*	36	+ 50.0	
	M	129	214	0.17	31	25	- 19.4	
	M	114	304	0.27	34	22	- 35.3	
Average		120.4	330.1	0.28	26.0	26.0	- 4.9	

Table X (contd.)

1	2	3	4	5	6	7	8	9
6	M	143	622	0.43	27	39	+ 44.4	
	F	101	561	0.56	29	36	+ 24.1	
	F	105	329	0.31	28	26	- 7.2	q = 24.1%
	F	121	434	0.36	21*	22	+ 4.8	
	M	139	419	0.30	27	21	- 22.2	
	M	103	331	0.32	21*	30	+ 42.9	
	F	85	361	0.42	21*	20	- 4.8	
	M	96	477	0.50	21*	26	+ 23.8	t = $\gg$10%
	F	72	316	0.44	23*	22	- 4.4	
	M	89	255	0.29	22*	31	+ 40.9	
Average		105.4	410.5	0.39	24.0	27.3	+ 14.3	
12	M	113	323	0.29	28	36	+ 28.6	
	F	103	354	0.34	26	30	+ 15.4	
	M	119	445	0.37	25*	29	+ 16.0	q = 13.7%
	M	90	329	0.37	23*	26	+ 13.0	
	M	100	548	0.55	21*	23	+ 9.5	
	M	104	223	0.21	29	31	+ 6.9	t = $>$10%
	M	116	485	0.42	24*	34	+ 41.7	
	F	112	486	0.43	31	30	- 3.2	
Average		107.1	386.6	0.37	25.8	28.6	+ 16.0	
24	F	90	186	0.21	35	46	+ 31.4	
	M	114	764	0.67	30*	45	+ 50.0	
	F	92	384	0.42	39	50	+ 28.2	q = 33.0%
	M	113	470	0.41	22*	44	+ 100	
	F	84	536	0.64	36	39	+ 8.3	
	M	102	375	0.37	32	28	- 12.5	
	M	105	432	0.41	28*	40	+ 42.9	t = 2-5%
	F	106	538	0.51	32	40	+ 25.0	
Average		100.8	460.6	0.46	30.5	41.5	+ 34.2	
48	M	123	418	0.34	28*	32	+ 14.3	
	F	106	665	0.63	18*	25	+ 38.9	q = 16.0%
	M	126	695	0.55	33	35	+ 6.1	
	F	95	761	0.80	33	43	+ 30.3	
	M	133	642	0.48	27*	37	+ 37.0	t = 2-5%
	F	100	646	0.65	28*	43	+ 53.6	
	M	117	504	0.43	30	34	+ 13.3	
	F	112	492	0.44	31	37	+ 19.4	
Average		114.0	602.9	0.54	28.5	34.5	+ 26.6	

Table X (contd.)

1	2	3	4	5	6	7	8	9
72	M	127	617	0.49	22*	28	+ 36.4	
	F	99	380	0.38	34	31	- 8.8	σ = 31.2%
	M	135	832	0.62	27*	34	+ 25.9	
	M	131	510	0.39	43	30	- 30.2	t = $\gg$10%
	M	102	542	0.53	30*	45	+ 50.0	
	M	131	481	0.37	35	32	- 8.6	
Average		125.3	560.3	0.46	31.8	33.3	+ 10.8	

* These values were used as small initial values on Graph VIII.

$\not$ Percentage injury = $\frac{\text{Sample weight}}{\text{Body weight}}$ x 100.

Table XI

Skin histamine values for 330 day-old rats before and after remote injury

1	2	3	4	5	6	7	8	9
Period after operation (hours)	Sex	Weight (g.)	Sample weight (mg.)	Percentage Injury	Initial value (microg./g.)	Final value (microg./g.)	Percentage change	Statistical Data
1	F	184	1210	0.66	12	12	0	
	M	350	1380	0.39	7	9	+ 28.6	σ = 15.9%
	M	335	925	0.28	9	8	- 11.2	
	F	185	791	0.43	17	17	0	t = $\gg$10%
	F	193	542	0.28	18	16	- 11.1	
	F	200	736	0.37	21	16	- 23.8	
	M	289	641	0.22	17	18	+ 5.9	
	M	330	565	0.17	20	22	+ 10.0	
Average		-	849	0.35	15.1	14.8	- 0.2	
4	M	360	1030	0.29	11	12	+ 9.1	
	F	180	917	0.51	13	16	+ 23.1	σ = 11.2%
	M	318	978	0.31	19	17	- 10.5	
	F	189	884	0.47	17	18	+ 5.9	t = $\gg$10%
	F	230	600	0.26	10	10	0	
	F	207	580	0.28	21	25	+ 19.0	
	F	202	629	0.31	22	20	- 9.1	
	M	300	629	0.21	23	26	+ 13.0	
	M	304	827	0.27	22	25	+ 13.6	
Average		-	786	0.32	17.6	18.8	+ 7.1	
8	F	170	826	0.49	13	13	0	
	M	330	1380	0.42	12	11	- 8.4	
	M	342	530	0.16	11	13	+ 18.2	σ = 17.5%
	F	195	630	0.32	19	15	- 21.0	
	M	313	665	0.21	13	11	- 15.4	t = $\gg$10%
	F	207	735	0.36	21	20	- 4.8	
	F	193	569	0.29	21	28	+ 33.3	
	M	310	734	0.24	24	25	+ 4.2	
Average		-	759	0.31	16.8	17.0	+ 0.8	

Table XI (contd.)

1	2	3	4	5	6	7	8	9
12	F	169	748	0.44	12	16	+ 33.3	
	M	307	1330	0.43	16	21	+ 31.3	σ = 13.1%
	M	339	795	0.23	11	11	0	
	F	195	825	0.42	16	20	+ 25.0	
	M	315	586	0.19	11	14	+ 27.3	t = 2-5%
	F	214	463	0.22	17	24	+ 41.2	
	F	192	571	0.30	21	23	+ 9.5	
	M	327	815	0.25	20	23	+ 15.0	
	M	320	1240	0.39	19	25	+ 31.6	
Average		-	819	0.32	15.9	19.7	+ 23.8	
24	F	188	1630	0.87	12	15	+ 25.0	
	M	325	1040	0.32	16	17	+ 6.3	
	M	290	882	0.30	17	18	+ 5.9	σ = 13.9%
	F	229	521	0.23	8	10	+ 25.0	
	M	330	710	0.22	9	12	+ 33.3	
	F	210	687	0.33	18	24	+ 33.3	t = 5-10%
	F	211	1040	0.49	18	26	+ 44.4	
	M	338	930	0.28	19	21	+ 10.5	
	M	273	892	0.33	20	22	+ 10.0	
Average		-	926	0.37	15.2	18.3	21.5	
48	M	347	1620	0.47	20	19	- 5.0	
	F	186	606	0.33	15	12	- 20.0	σ = 14.5%
	F	190	743	0.39	12	10	- 16.7	
	M	380	716	0.19	18	16	- 11.1	
	F	210	756	0.36	16	17	+ 6.3	t = $\gg$10%
	F	220	774	0.35	17	20	+ 17.6	
	M	315	914	0.29	19	20	+ 5.3	
	M	274	1078	0.39	30	35	+ 16.7	
Average		-	901	0.35	18.4	18.6	- 0.9	
72	M	281	1120	0.40	13	11	- 15.4	
	M	302	1520	0.50	8	8	0	σ = 27.1%
	F	180	504	0.28	16	14	- 12.5	
	F	185	470	0.25	18	20	+ 11.1	
	M	370	621	0.17	10	8	- 20.0	t = $\gg$10%
	F	212	841	0.40	14	23	+ 64.3	
	M	301	377	0.13	23	25	+ 8.7	
	M	301	581	0.19	20	24	+ 20.0	
Average		-	754	0.28	15.5	16.6	+ 7.0	

Table XII

Skin histamine values of 21 - 25 day-old adrenalectomised rats before and after remote injury

1	2	3	4	5	6	7	8	9*
Period after operation (hours)	Initial value (microg./g.)	Final value (microg./g.)	Age (days) at start of experiment	Litter size	Average normal change (microg./g.) Graph III	Percentage change uncorrected	Percentage change corrected	Statistical data
6	44.4	46.7	23	5	+ 2.6	+ 5.2	- 0.7	$q\,q_u$ 14.4
	47.6	58.5	22	2	- 0.5	+22.9	+24.0	q_c 15.9
	29.7	32.9	23	2	+ 2.6	+10.8	+ 2.0	$t_a \gg 10\%$
	31.5	44.7	21	3-6	+ 1.6	+41.9	+36.8	$t_b \gg 10\%$
	32.8	37.1	21	3-6	+ 1.6	+13.1	+ 8.2	
	Average					+18.8	+14.1	
12	46.1	62.0	25	6-8	- 2.0	+34.5	+38.9	
	45.9	45.7	25	6-8	- 2.0	- 0.4	+ 3.9	
	Average					+17.1	+21.4	
24	70.8	62.5	22	12	-11.4	-11.7	+ 4.4	$q\,q_u$ 19.9
	70.4	78.4	22	12	-11.4	+11.4	+27.6	q_c 22.5
	48.8	69.6	22	13	-11.4	+42.7	+66.0	t_a 1-0.1%
	65.7	76.5	22	13	-11.4	+16.4	+33.8	t_b 1-2%
	64.3	67.1	22	13	-11.4	+ 4.3	+22.1	
	Average					+12.6	+30.8	
48	37.3	67.2	23	12	+ 5.8	+80.2	+64.7	$q\,q_u$ 46.1
	64.1	45.2	23	12	+ 5.8	-28.7	-37.7	q_c 50.1
	53.0	93.1	25	9-10	- 8.6	+75.8	+92.0	$t_a \gg 10\%$
	58.9	55.4	24	8	- 2.0	- 4.2	- 2.5	$t_b > 10\%$
	58.5	56.2	22	Large	- 8.0	- 3.9	+ 9.7	
	48.1	48.7	21	Large	+ 9.2	+ 1.2	-17.9	
	Average					+20.1	+18.1	

* See Table IX for explanation of symbols

Table XIII

Skin histamine values of 50-day-old adrenalectomised rats before and after remote injury

Time after injury (hours)	Sex	Body weight (g.)	Sample weight (mg.)	% Injury	Initial value (microg./g.)	Final value (microg./g.)	% Change	Statistical Evaluation
3	M	135	309	.23	26	37	+42.3	"t"
	F	108	408	.38	30	29	- 3.3	≫10%
	M	119	645	.54	22	36	+63.9	q =
	M	124	361	.29	27	19	-29.7	42.4
Average		121.5	430.8	.36	26.3	30.3	+18.2	
6	M	149	538	.36	37	33	-10.8	
	F	115	426	.37	24	37	+54.2	"t"
	F	110	366	.33	27	27	0	≫10%
	M	137	467	.34	25	27	+8	
	F	86	392	.46	17	22	+29.4	q =
	M	99	352	.36	25	22	-12	22.5
	F	82	277	.34	25	31	+24	
	M	85	202	.24	22	23	+4.5	
Average		107.9	377.5	.35	25.3	27.8	+12.2	
12	M	114	372	.33	25	32	+28	"t"
	F	104	352	.34	23	22	-4.3	≫10%
	M	110	509	.46	19	27	+42.1	q =
	M	94	399	.42	27	26	-3.7	23.3
Average		105.5	408	.39	23.5	26.8	+15.5	

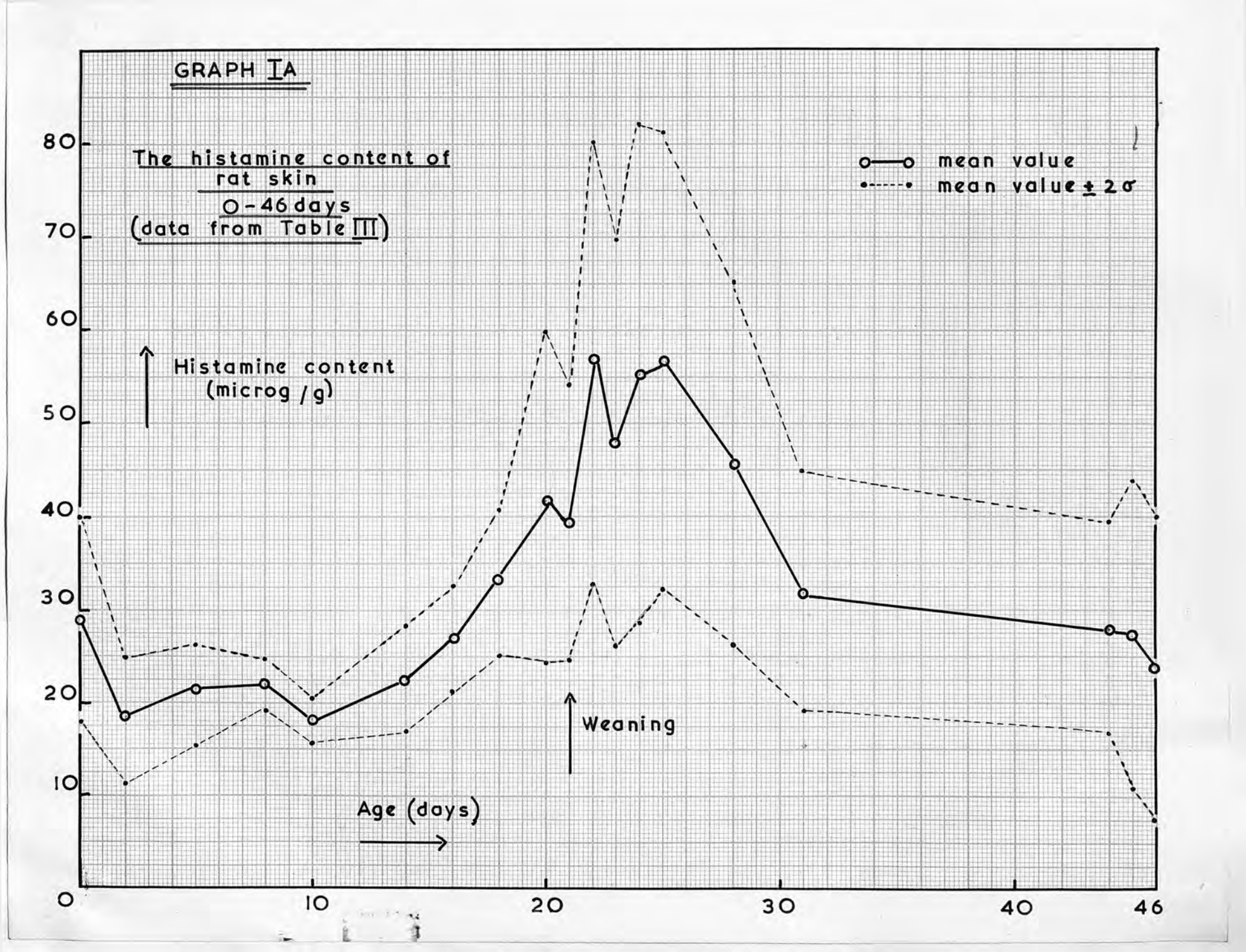
Time of second sample (hours)	Sex	Body weight (g.)	Sample weight (mg.)	% Injury	Initial value (microg./g.)	Final value (microg./g.)	% Change	Statistical Evaluation
24	F	91	551	.61	33	45	+36.4	"t"
	F	91	550	.60	40	54	+35	=1-.1%
	M	106	697	.66	25	59	+136	q =
	M	92	752	.82	33	43	+30.3	51.1
Average		95	637.5	.67	32.8	50.3	+59.4	
48	M	122	688	.56	27	29	+7.4	
	F	101	862	.85	28	33	+17.9	"t"
	M	126	692	.55	29	37	+27.6	=2-5%
	F	95	649	.68	41	50	+22	q =
	M	121	829	.69	26	56	+115.4	43.9
Average		113	744	.67	30.2	41	+38.1	
72	M	128	487	.38	24	34	+41.7	
	F	103	634	.62	29	33	+13.8	"t"
	M	126	592	.47	28	28	0	=2-5%
	M	128	691	.54	20	27	+35	q =
	M	122	776	.64	18	34	+88.9	29.9
Average		121.4	618	.53	23.8	31.2	+35.9	

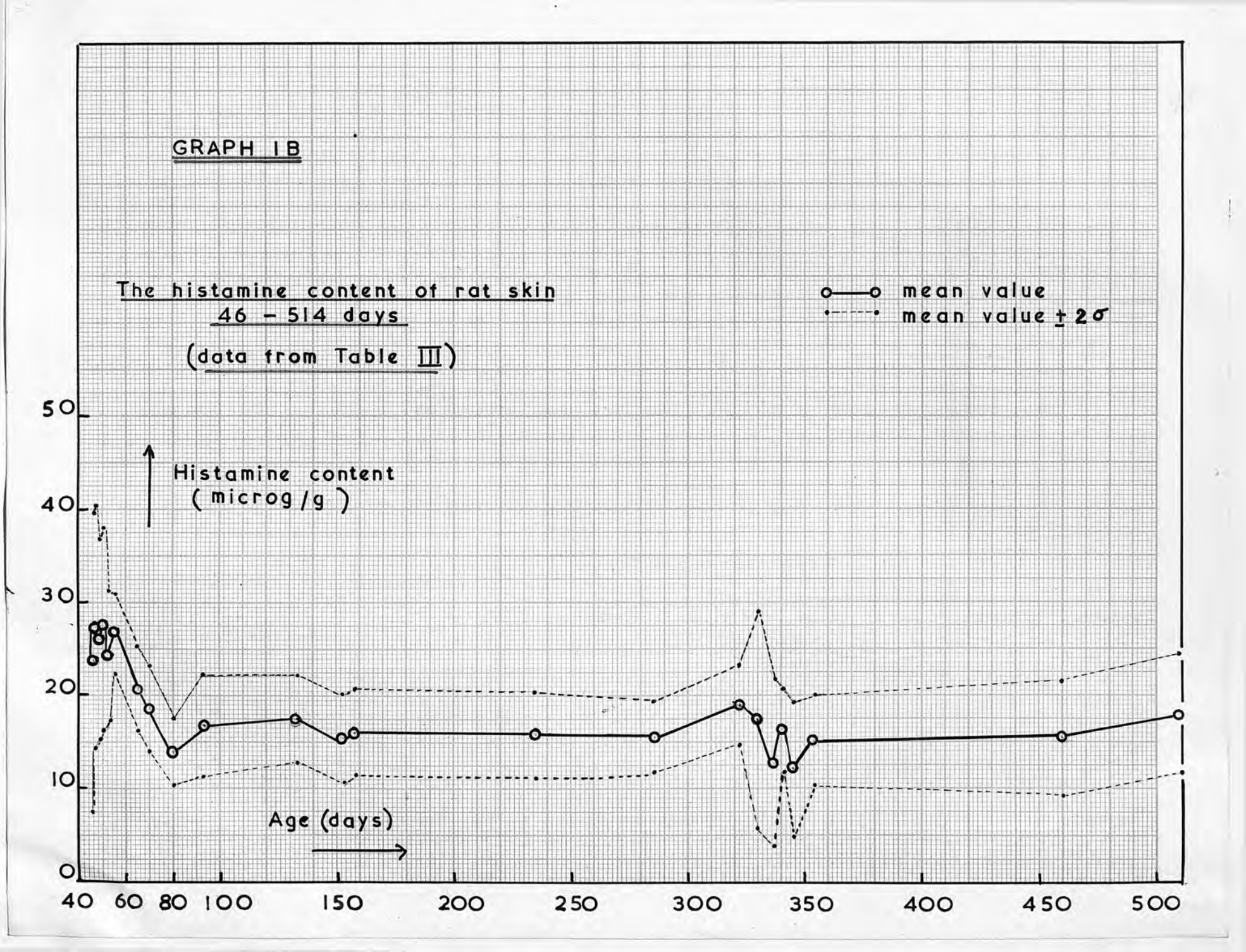
* % Injury = $\frac{\text{Weight of Sample x 100}}{\text{Body weight}}$

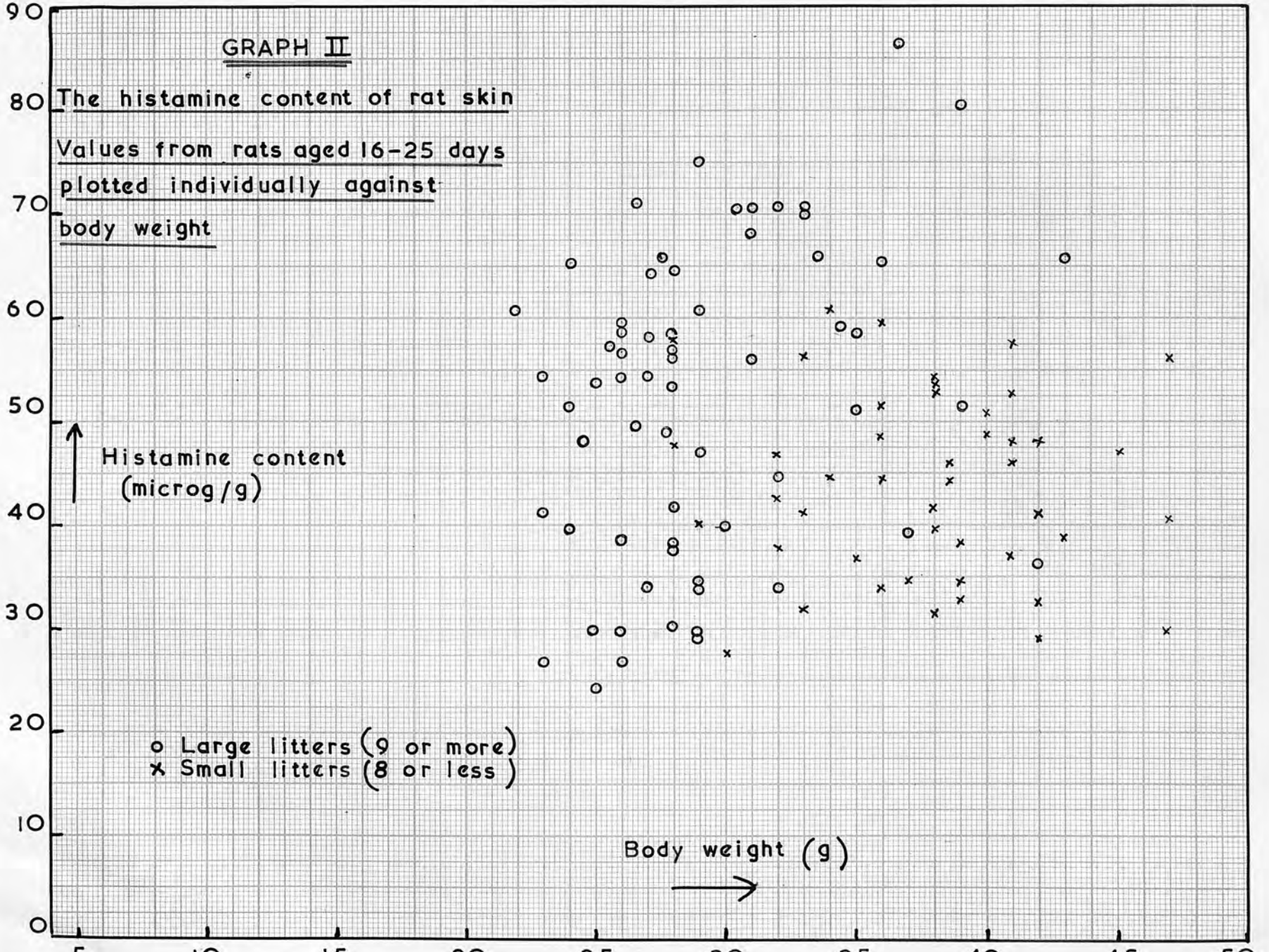
Table XIV

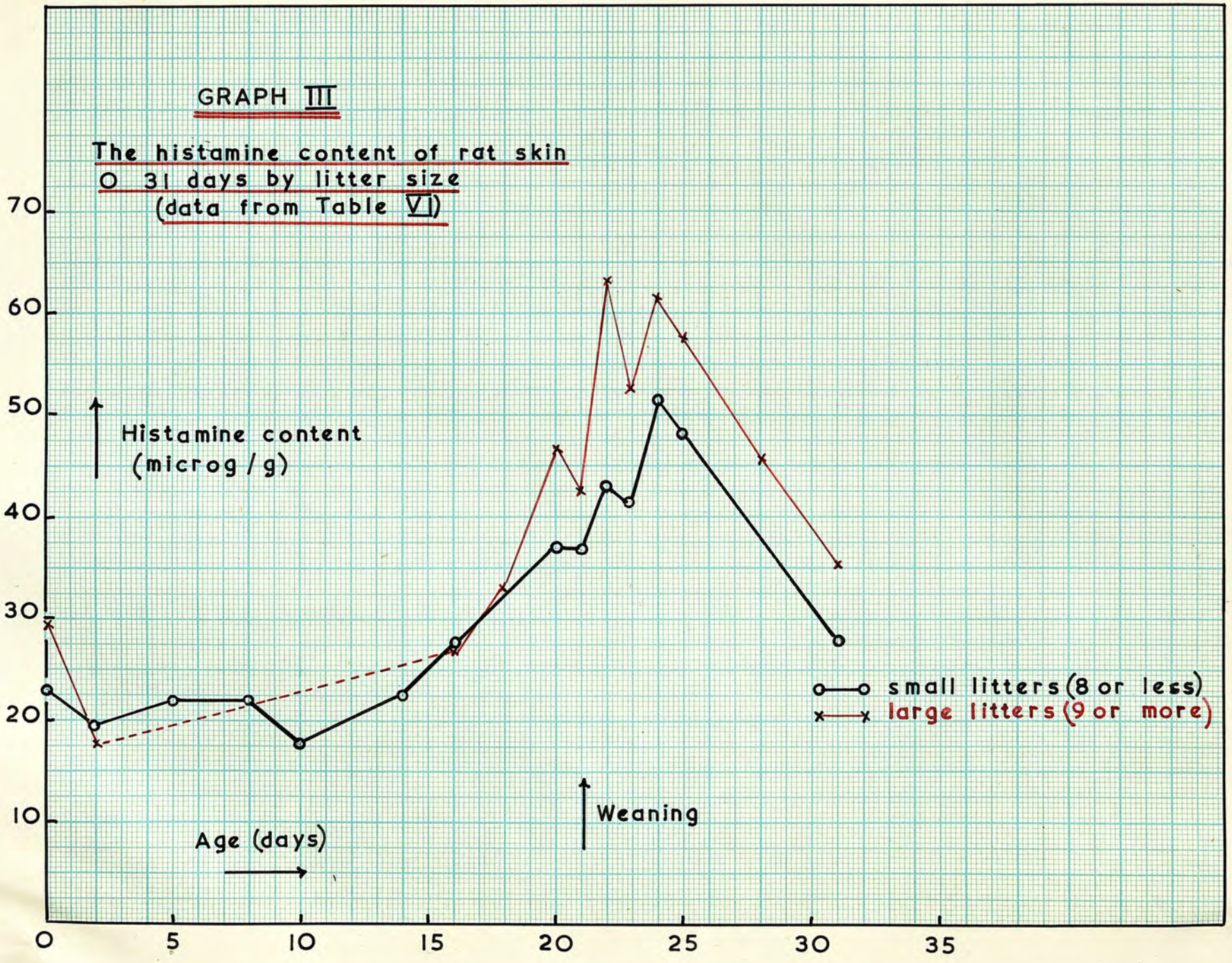
Skin histamine values of samples taken within 3 minutes of each other.

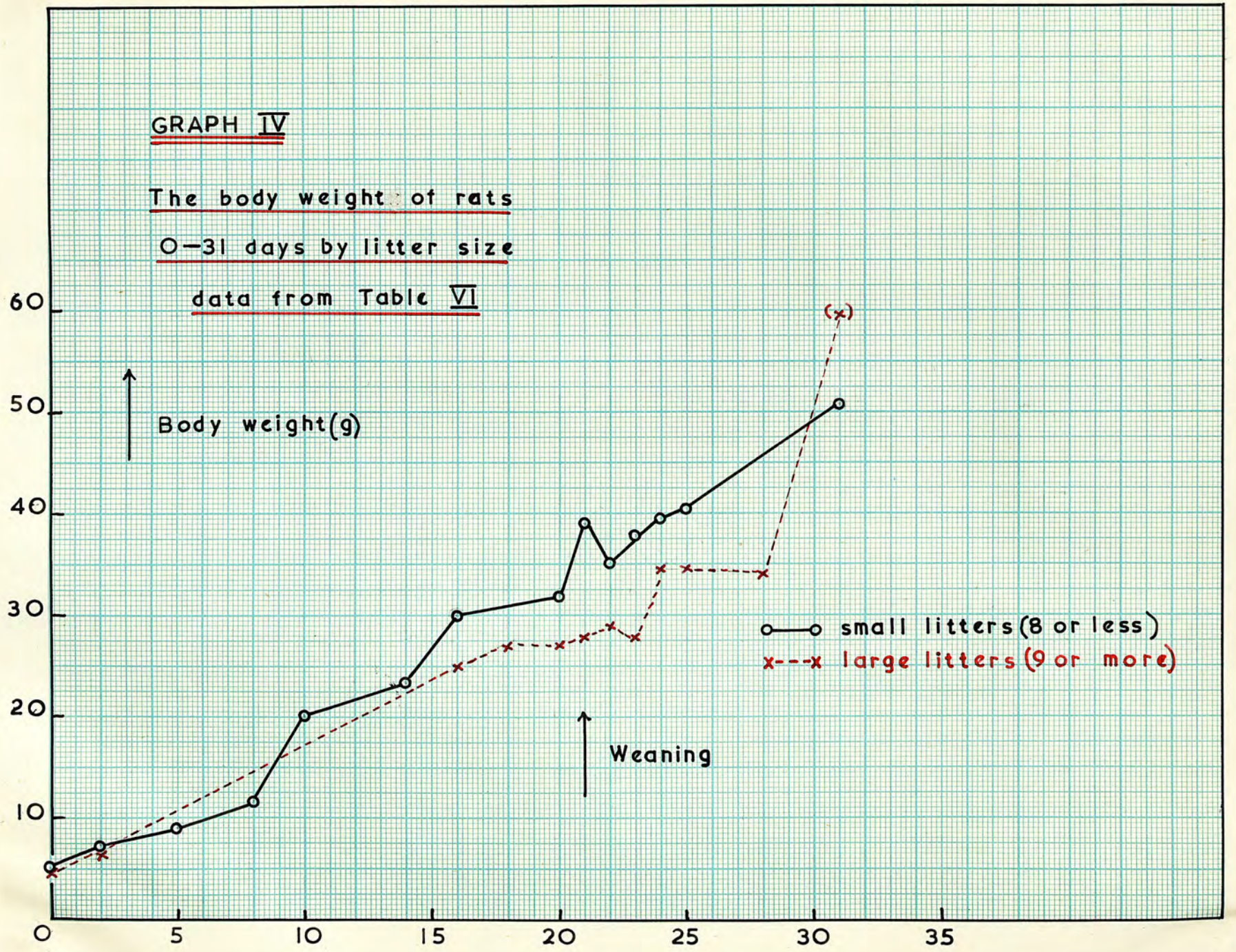
Sex	Age (days)	Body Weight (g.)	Initial Value (microg./g.)	Final Value (microg./g.)	Percentage Change	
F	28	37	57.9	54.4	- 6.0	
M	28	34	54.6	53	- 2.9	
M	28	33	30.2	54.8	+ 81.5	
F	28	32	48.8	39.4	- 19.3	
F	158	172	17.4	16.5	- 5.2	
F	158	179	12.8	13.6	+ 6.3	
M	158	307	14.3	13	- 9.1	
M	158	319	17	19.8	+ 16.5	
F	235	209	22.2	16.2	- 27.0	
F	235	204	13.8	14.8	+ 7.2	
M	235	272	11.8	15.1	+ 28.0	
M	286	353	13.4	16.2	+ 20.9	
M	286	342	13.4	11.9	- 11.2	
F	286	220	15.8	16.3	+ 3.1	
F	286	194	18.4	17.3	- 6.0	
F	286	214	16.4	15.7	- 4.3	
Averages			23.64	24.25	+ 4.5	24.9

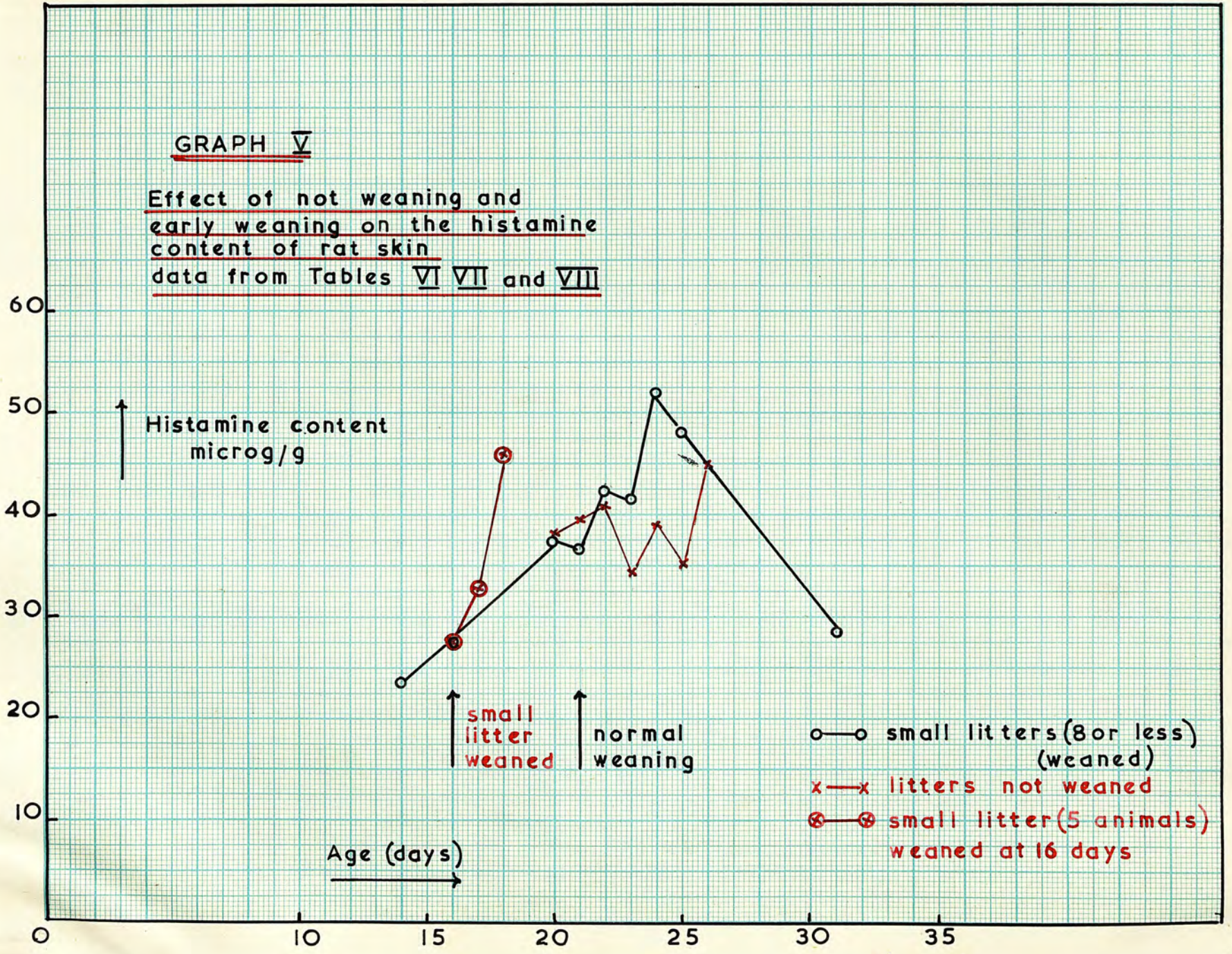

GRAPH IA
The histamine content of rat skin
0-46 days
(data from Table III)
mean value
mean value ± 2σ
Histamine content (microg / g)
Weaning
Age (days)
0
10
20
30
40
46
10
20
30
40
50
60
70
80

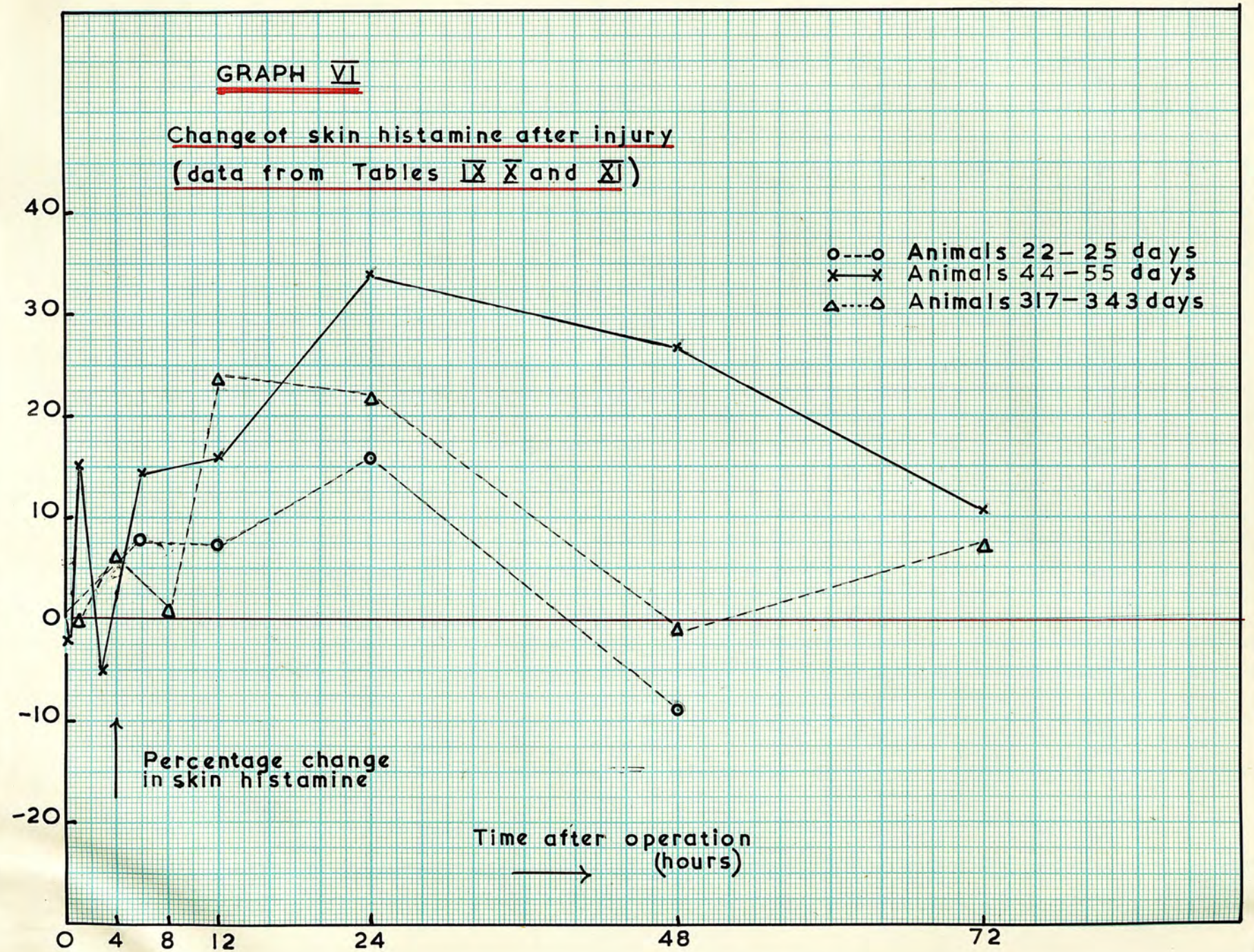

GRAPH 1B
The histamine content of rat skin
46 - 514 days
(data from Table III)
mean value
mean value ± 2σ
Histamine content
(microg / g)
Age (days)
0
10
20
30
40
50
40
60
80
100
150
200
250
300
350
400
450
500


GRAPH II
The histamine content of rat skin
Values from rats aged 16–25 days
plotted individually against
body weight
Histamine content
(microg/g)
o Large litters (9 or more)
x Small litters (8 or less)
Body weight (g)
0
10
20
30
40
50
60
70
80
90
5
10
15
20
25
30
35
40
45
50


GRAPH III
The histamine content of rat skin
0 31 days by litter size
(data from Table VI)
70
60
50
40
30
20
10
Histamine content
(microg / g)
small litters (8 or less)
large litters (9 or more)
Weaning
Age (days)
0
5
10
15
20
25
30
35

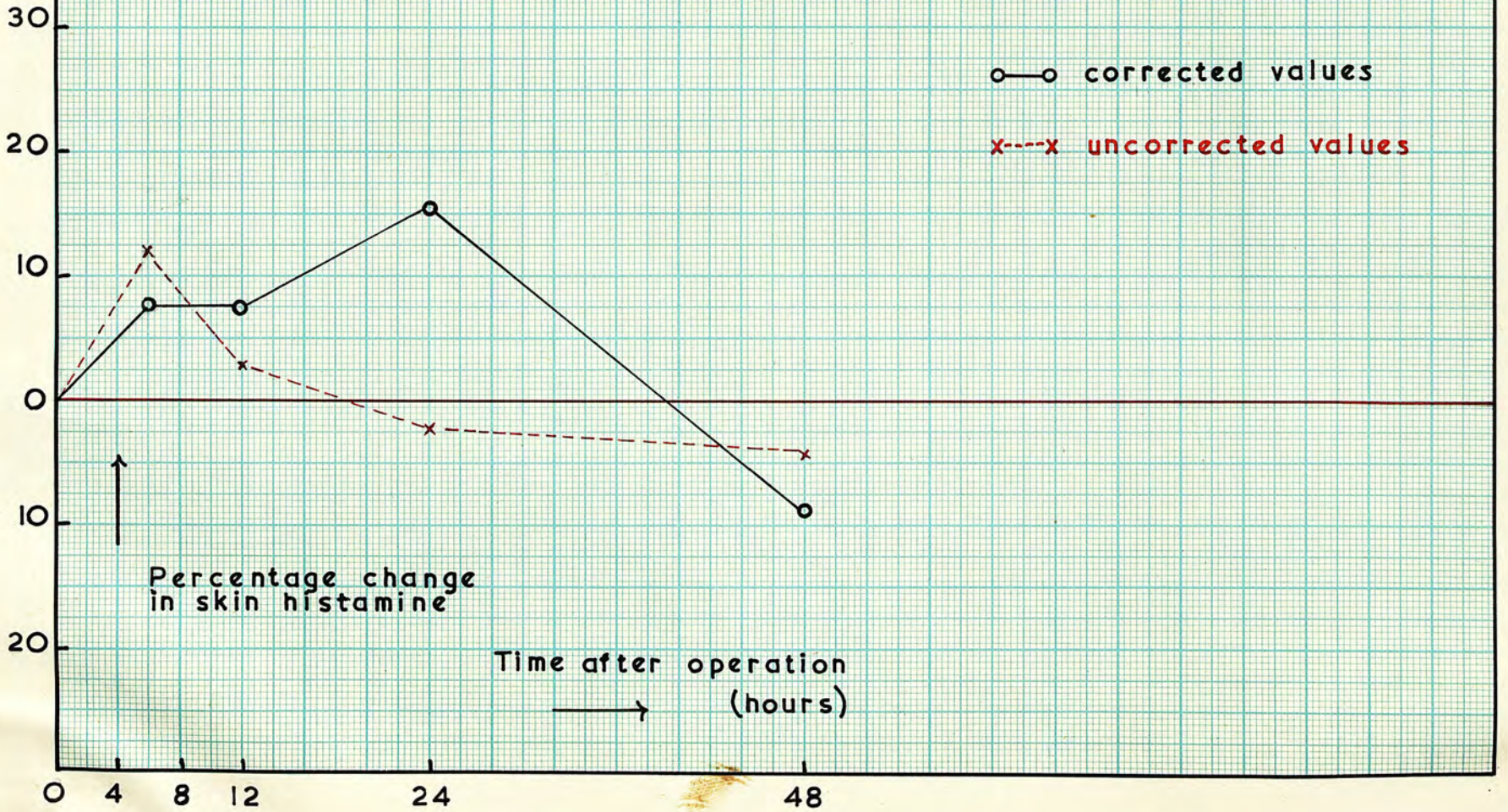

GRAPH IV
The body weight of rats
0—31 days by litter size
data from Table VI
Body weight(g)
60
50
40
30
20
10
0
5
10
15
20
25
30
35
(x)
small litters (8 or less)
x---x large litters (9 or more)
Weaning

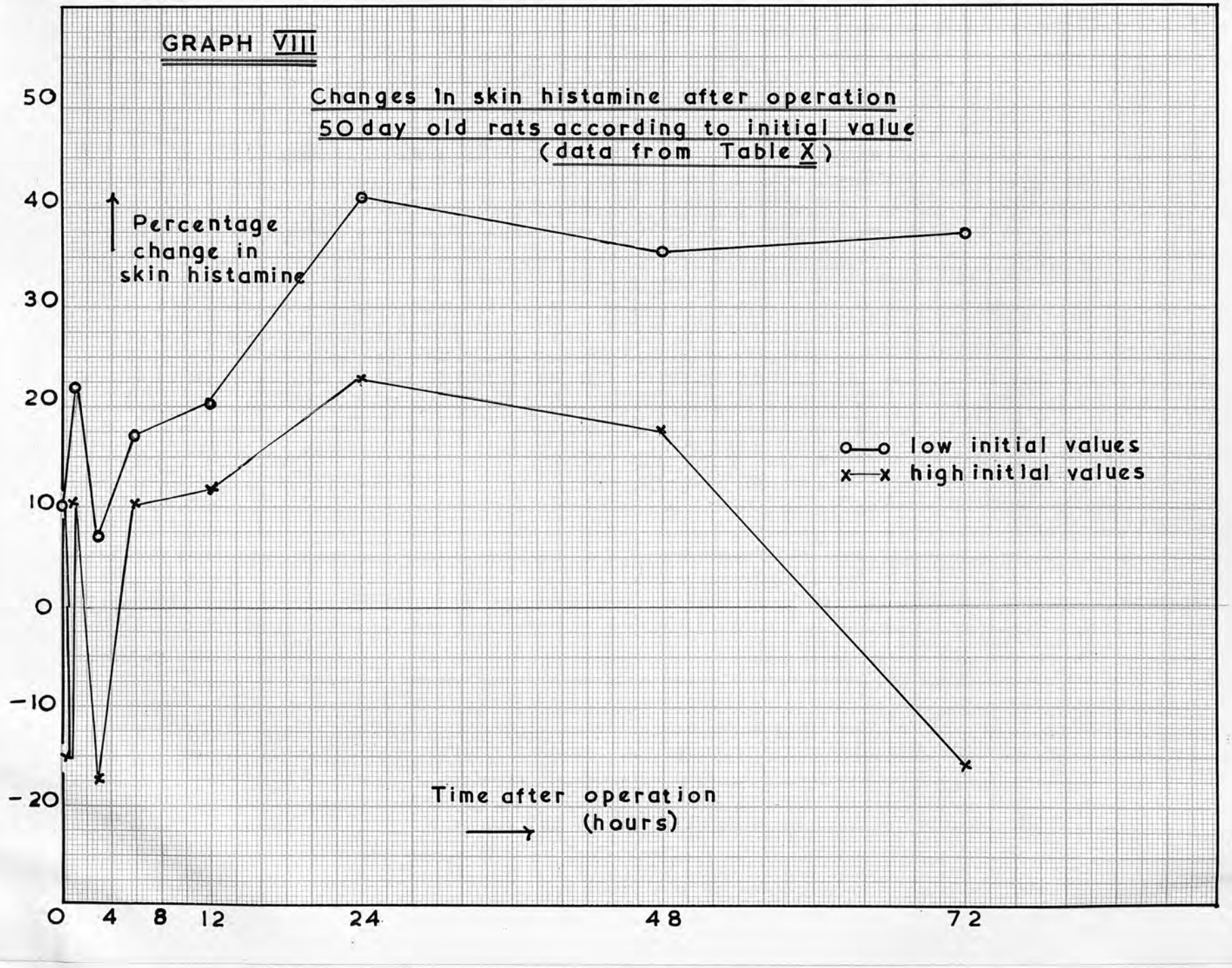

GRAPH V
Effect of not weaning and early weaning on the histamine content of rat skin
data from Tables VI VII and VIII
Histamine content microg/g
60
50
40
30
20
10
0
10
15
20
25
30
35
Age (days)
small litter weaned
normal weaning
small litters (8 or less) (weaned)
litters not weaned
small litter (5 animals) weaned at 16 days

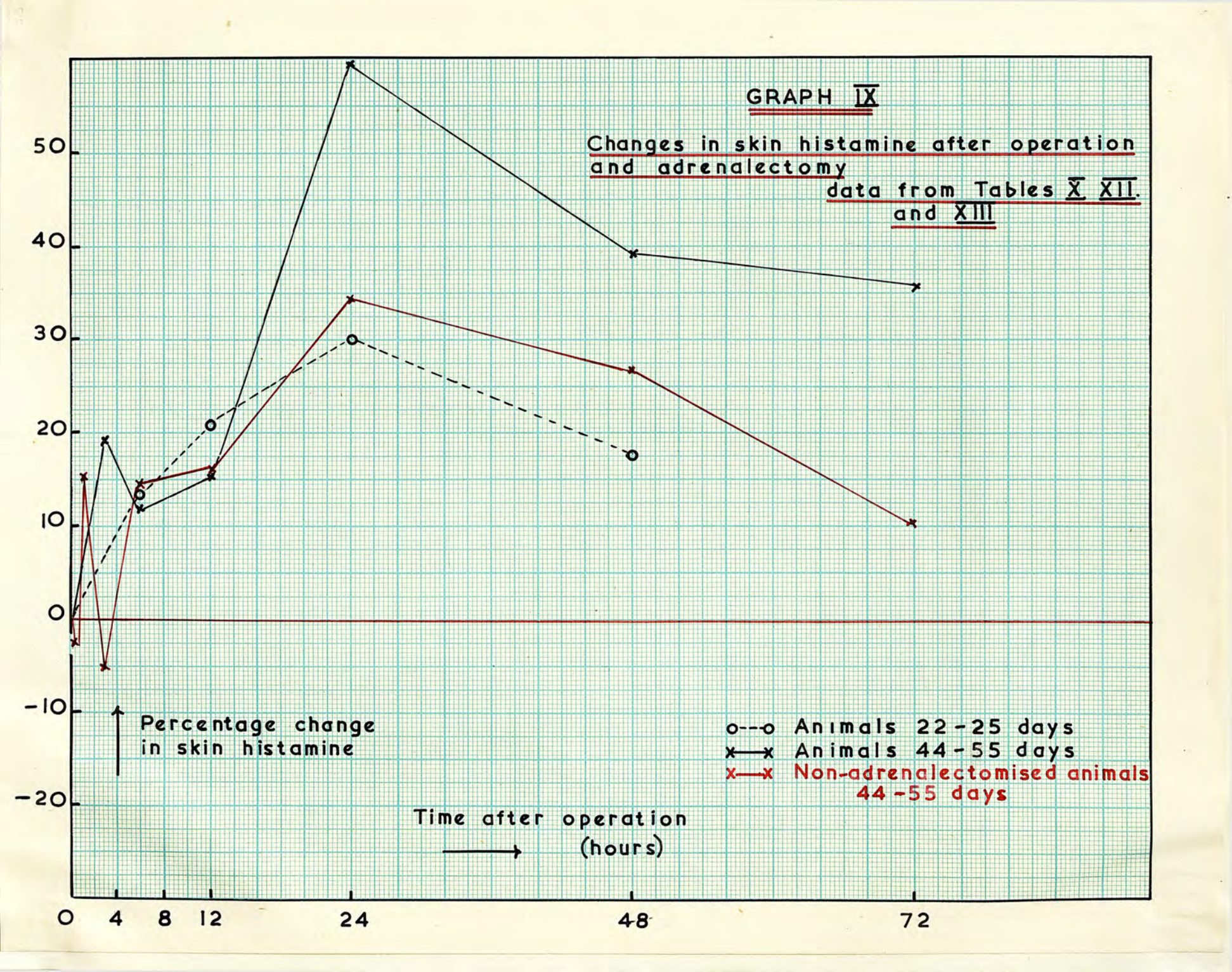

GRAPH VI
Change of skin histamine after injury
(data from Tables IX X and XI)
40
30
20
10
0
-10
-20
o---o Animals 22–25 days
x——x Animals 44–55 days
Δ····Δ Animals 317–343 days
Percentage change in skin histamine
Time after operation (hours)
0
4
8
12
24
48
72

GRAPH VII

Effect of correcting operated skin histamine changes to allow for the normal age change in operated animals (data from Table IX)

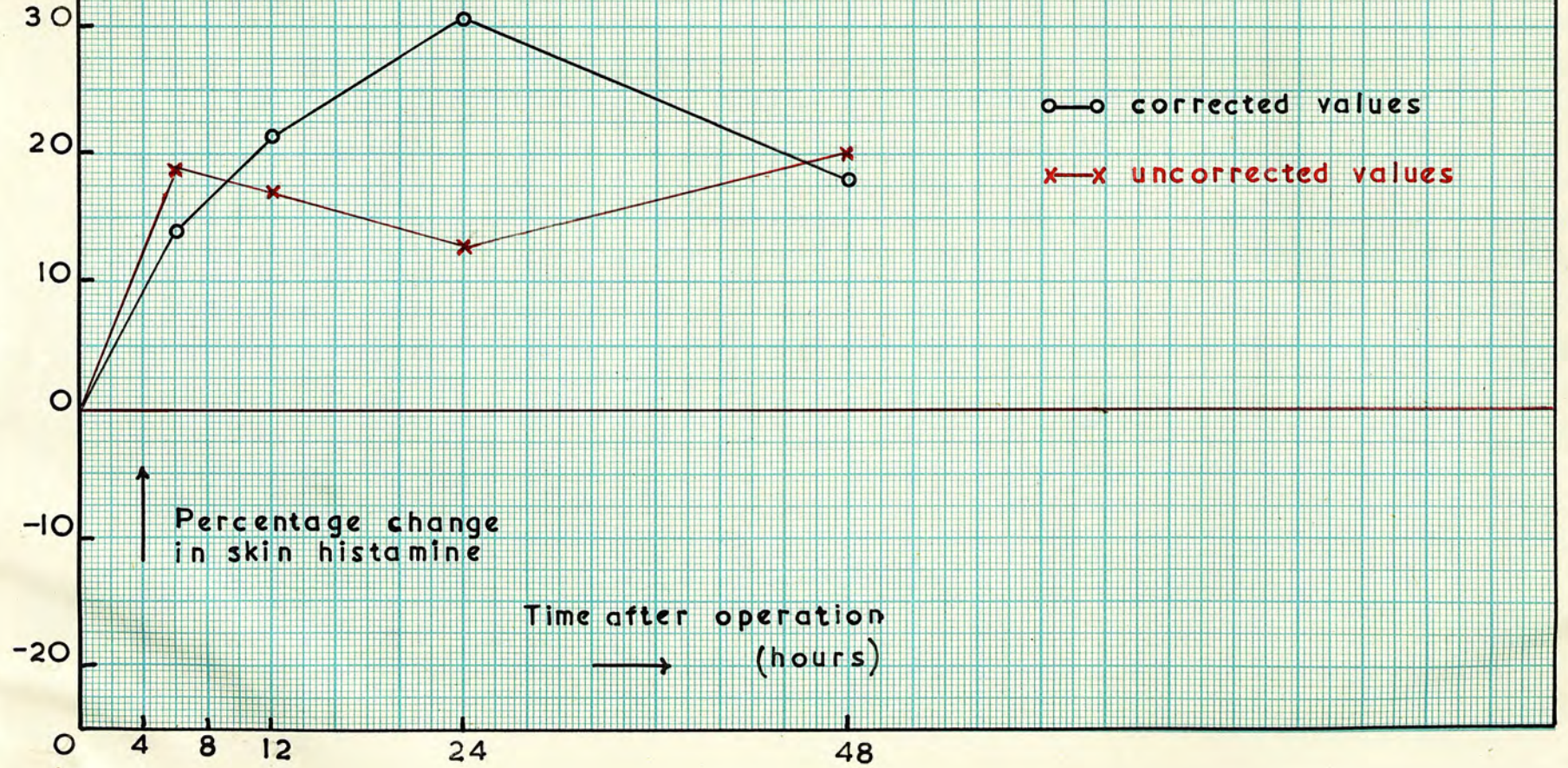


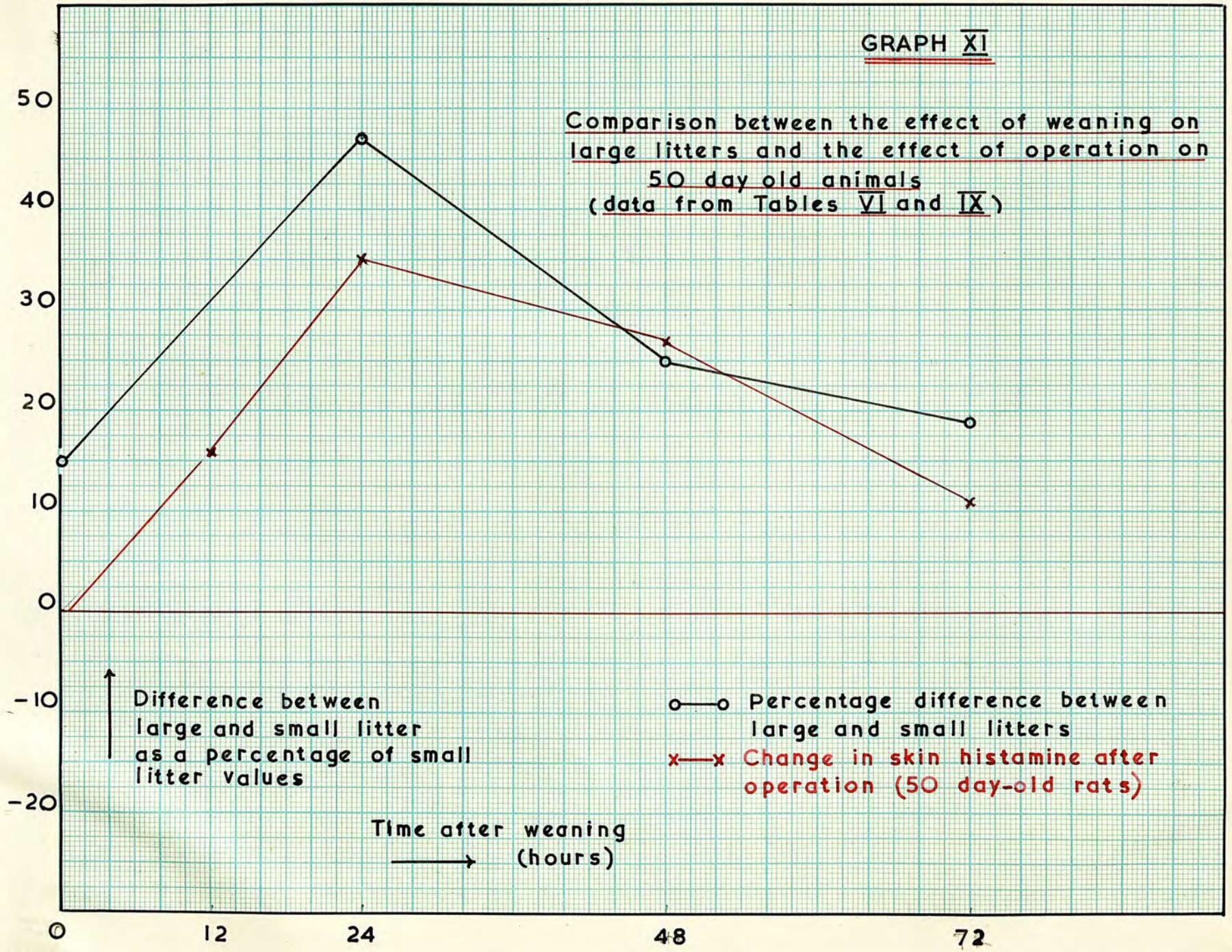

GRAPH VIII
Changes in skin histamine after operation
50 day old rats according to initial value
(data from Table X)
Percentage change in skin histamine
low initial values
high initial values
Time after operation (hours)
50
40
30
20
10
0
-10
-20
0
4
8
12
24
48
72


GRAPH IX
Changes in skin histamine after operation and adrenalectomy
data from Tables X XII. and XIII
50
40
30
20
10
0
-10
-20
Percentage change in skin histamine
o--o Animals 22-25 days
x—x Animals 44-55 days
x—x Non-adrenalectomised animals 44-55 days
Time after operation (hours)
0
4
8
12
24
48
72

GRAPH X

Effect of correcting 'operated' skin histamine changes to allow for the normal age change in adrenalectomised animals (data from Table XII)




GRAPH XI
Comparison between the effect of weaning on large litters and the effect of operation on 50 day old animals
(data from Tables VI and IX)
50
40
30
20
10
0
-10
-20
Difference between large and small litter as a percentage of small litter values
Time after weaning (hours)
0
12
24
48
72
Percentage difference between large and small litters
Change in skin histamine after operation (50 day-old rats)